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(71) Applicants *(for all designated States except US)*: CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE [-/-]; 3, rue Michel-Ange, F-75794 Paris Cedex 16 (FR). THE UAB RESEARCH FOUNDATION [US/US]; Room 113, MJH, 1825 University Boulevard, Birmingham, AL 35294-2010 (US). EMORY UNIVERSITY [US/US]; 1380 S. Oxford Road, Atlanta, GA 30322 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GOSSELIN, Gilles [FR/FR]; 82, rue Calvin, 400, avenue Paul Rimbaud, F-34080 Montpellier (FR). IMBACH, Jean-Louis [FR/FR]; Impasse des Luquesop, 1108 Reu las Sorbes, F-34000 Montpellier (FR). SOMMADOSSI, Jean-Pierre [US/US]; 5075 Greystone Way, Birmingham, AL 3524 (US). SCHINAZI, Raymond, F. [US/US]; 1524 Regency Walk Drive, Decatur, GA 30033 (US).

(74) Agent: KNOWLES, Sherry; King & Spalding, 191 Peachtree Street, Atlanta, GA 30303-1763 (US).

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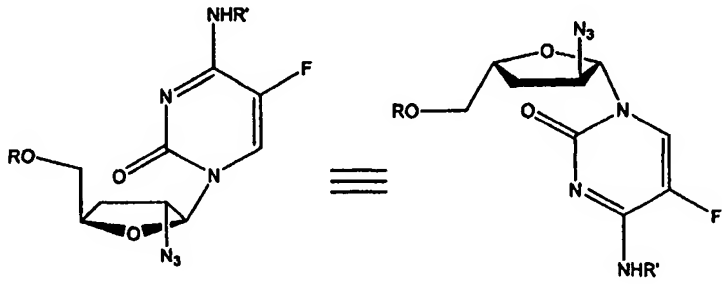

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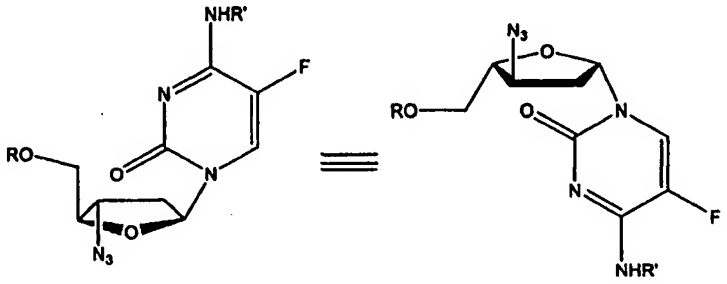

(II)

(57) Abstract

Compounds and pharmaceutical compositions active againt HIV are provided, as is a method for the treatment of hepatitis B virus infection in humans and other host animals is provided comprising administering an effective amount of a β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine of formulae (I) and (II) wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

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β-L-2'-Deoxy-Nucleosides for the Treatment of HIV Infection

Background of the Invention

This invention is in the area of methods for the treatment of human immunodeficiency virus (also referred to as "HIV") that includes administering to a host in need thereof, either alone or in combination, an effective HIV-treatment amount of one or more of the active compounds disclosed herein, or a pharmaceutically acceptable prodrug or salt of one of these compounds.

In 1981, acquired immune deficiency syndrome (AIDS) was identified as a disease that severely compromises the human immune system, and that almost without exception leads to death. In 1983, the etiological cause of AIDS was determined to be the human immunodeficiency virus (HIV).

In 1985, it was reported that the synthetic nucleoside 3'-azido-3'-deoxythymidine (AZT) inhibits the replication of human immunodeficiency virus. Since then, a number of other synthetic nucleosides, including (-)-β-2',3'-dideoxy-3'-thiacytidine (3TC), β-2',3'-dideoxy-3'-thia-5-fluorocytidine (FTC), 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), and 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), have been proven to be effective against HIV. After cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides are incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group. They can also inhibit the viral enzyme reverse transcriptase.

The success of various synthetic nucleosides in inhibiting the replication of HIV *in vivo* or *in vitro* has led a number of researchers to design and test nucleosides that substitute a heteroatom for the carbon atom at the 3'-position of the nucleoside. European Patent Application Publication No. 0 337 713 and U.S. Patent No. 5,041,449, assigned to BioChem Pharma, Inc., disclose racemic 2-substituted-4-substituted-1,3-dioxolanes that exhibit antiviral activity. U.S. Patent No. 5,047,407 and European Patent Application Publication No. 0 382 526, also assigned to BioChem Pharma, Inc., disclose that a number of racemic 2-substituted-5-substituted-1,3-oxathiolane nucleosides have antiviral activity, and specifically report that the racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane

(referred to as BCH-189) has approximately the same activity against HIV as AZT, with little toxicity. The (-)-enantiomer of the racemate BCH-189, known as 3TC, which is covered by U.S. Patent No. 5,539,116 to Liotta *et al.*, is currently sold for the treatment of HIV in humans in the U.S. in combination with AZT.

It has also been disclosed that cis-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC") has potent HIV activity. Schinazi, *et al.*, "Selective Inhibition of Human Immunodeficiency viruses by Racemates and Enantiomers of cis-5-Fluoro-l-[2-(Hydroxymethyl)-1,3-Oxathiolane-5-yl]Cytosine" *Antimicrobial Agents and Chemotherapy*, November 1992, pp. 2423-2431. See also U.S. Patent Nos. 5,210,085; 5,814,639; 5,728,575; 5,827,727; 5,914,331; WO 91/11186 and WO 92/14743.

WO 96/40164 filed by Emory University, UAB Research Foundation, and the Centre National de la Recherche Scientifique discloses a number of β-L-2',3'-dideoxynucleosides for the treatment of hepatitis B.

WO 95/07287 also filed by Emory University, UAB Research Foundation, and the Centre National de la Recherche Scientifique discloses 2' or 3' deoxy and 2',3'-dideoxy-β-L-pentofuranosyl nucleosides for the treatment of HIV infection.

WO96/13512 filed by Genencor International, Inc., and Lipitek, Inc., discloses the preparation of L-ribofuranosyl nucleosides as antitumor agents and virucides.

WO95/32984 discloses lipid esters of nucleoside monophosphates as immunosuppresive drugs.

DE4224737 discloses cytosine nucleosides and their pharmaceutical uses.

Tsai, et al., in Biochem. Pharmacol. 48(7), pages 1477-81, 1994 discloses the effect of the anti-HIV agent 2'-β-D-F-2',3'-dideoxynucleoside analogs on the cellular content of mitochondrial DNA and lactate production.

Galvez, J. Chem. Inf. Comput. Sci. (1994), 35(5), 1198-203 describes molecular computation of β-D-3'-azido-2',3'-dideoxy-5-fluorocytidine.

Mahmoudian, Pharm. Research 8(1), 43-6 (1991) discloses quantitative structure-activity relationship analyses of HIV agents such as β-D-3'-azido-2',3'-dideoxy-5-fluorocytidine.

U.S. Patent No. 5,703,058 discloses (5-carboximido or 5-fluoro)-(2',3'-unsaturated or 3'-modified) pyrimidine nucleosides for the treatment of HIV and HBV infection.

Lin, et al., discloses the synthesis and antiviral activity of various 3'-azido analogues of β-D-nucleosides in J. Med. Chem. 31(2), 336-340 (1988).

In light of the fact that acquired immune deficiency syndrome and AIDS-related complex have reached epidemic levels worldwide, and have tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat these diseases that have low toxicity to the host.

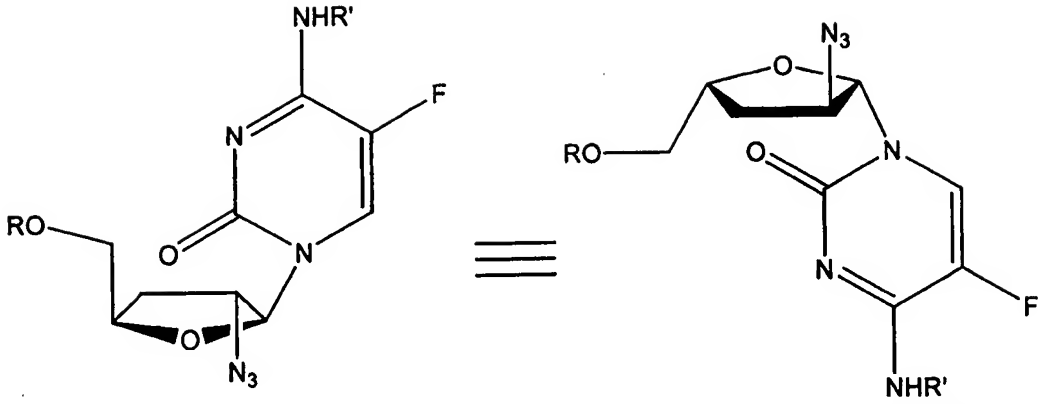
It is an object of the present invention to provide a compound and method for the treatment of human patients or other host animals infected with HIV.

Summary of the Invention

A method for the treatment of HIV infection in humans and other host animals is disclosed that includes administering an effective HIV-treatment amount to the host of a β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine nucleoside or a pharmaceutically acceptable salt, ester, or prodrug thereof, including a stabilized phosphate, administered either alone or in combination or alternation with another anti-HIV agent, optionally in a pharmaceutically acceptable carrier. In a preferred embodiment, the 2' or 3'-azido group is in the ribosyl configuration.

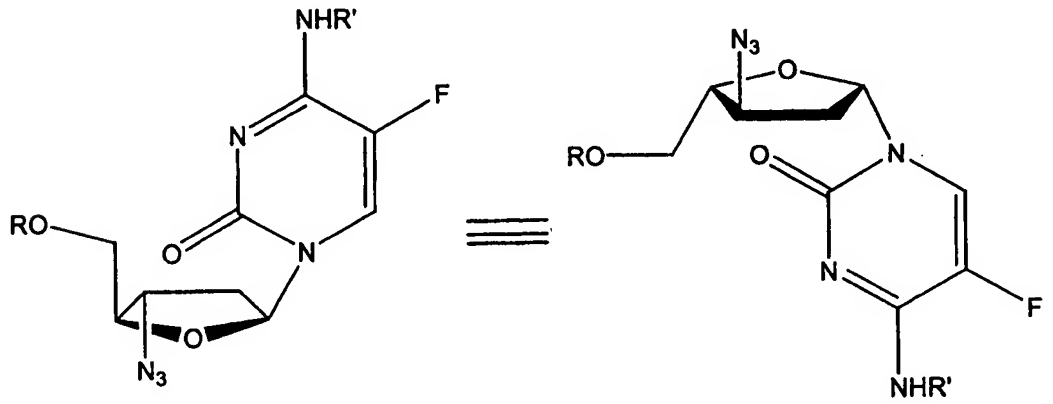
The disclosed β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine nucleosides, or pharmaceutically acceptable salts, esters, or prodrugs or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HIV infections and other related conditions such as Acquired Immune Deficiency Syndrome (AIDS), AIDS-Related Complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpurea and opportunistic infections. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

In one embodiment, the active compound is β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine (L-2'-A-5-FddC) or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), R' is H, acyl, or alkyl.

In another embodiment, the active compound is β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine (L-3'-A-5-FddC) or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

In another embodiment, the L-(2' or 3')-A-5-FddC nucleoside is administered in alternation or combination with one or more other compounds which exhibit activity against HIV, as described in more detail below. In general, during alternation therapy, an effective

dosage of each agent is administered serially, whereas in combination therapy, an effective dosage of two or more agents are administered together. The dosages will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. In one preferred embodiment, the compound is administered in combination with AZDU.

Brief Description of the Figures

Figure 1 is an illustration of a general reaction scheme for the stereospecific synthesis of 3'-substituted β-L-dideoxynucleosides.

Figure 2 is an illustration of a general reaction scheme for the stereospecific synthesis of 2'-substituted β-L-dideoxynucleosides.

Figure 3 is an illustration of one process for the preparation of β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine (L-3'-A-5-FddC).

Figure 4 is an illustration of one process for the preparation of β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytidine (L-2'-A-5-FddC).

Detailed Description of the Invention

A method for the treatment of HIV infection in humans and other host animals is disclosed that includes administering an effective amount of a β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosine nucleoside (referred to below as "L-(2' or 3')-A-5-FddC") or a pharmaceutically acceptable salt, ester, or prodrug thereof, including a stabilized phosphate, either alone or in combination or alternation with another anti-HIV agent, optionally in a pharmaceutically acceptable carrier.

The compounds described herein can be used to treat AIDS and AIDS-related conditions including Acquired Immune Deficiency Syndrome (AIDS), AIDS-Related Complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpurea and opportunistic infections. The method of the present invention includes the use of an L-(2' or 3')-A-5-FddC prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

As used herein, the term "substantially in the form of a single isomer" "substantially free of" or "substantially in the absence of" refers to a nucleoside that is at least approximately 95% in the designated stereoconfiguration.

The term alkyl, as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon of C_1 to C_{10}, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *t*-butyl, cyclobutyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The alkyl group can be optionally substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., "Protective Groups in Organic Synthesis," John Wiley and Sons, Second Edition, 1991. The term lower alkyl, as used herein, and unless otherwise specified, refers to a C_1 to C_4 ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, or t-butyl group.As used herein,

the term acyl refers to moiety of the formula -C(O)R', wherein R' is alkyl; aryl, alkaryl, aralkyl, heteroaromatic, alkoxyalkyl including methoxymethyl; arylalkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy, or the residue of an amino acid. The term acyl specifically includes but is not limited to acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic.

The L-(2' or 3')-A-5-FddC nucleoside can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. The nucleoside or its pharmaceutically acceptable prodrug can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base or acid. The ester or salt can be converted into the parent nucleoside, for example, by hydrolysis.

As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of the L-(2' or 3')-A-5-FddC that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with cations such as sodium, potassium, zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylene-diamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

The term prodrug, as used herein, refers to a compound that is converted into the nucleoside on administration in vivo, or that has activity in itself. Nonlimiting examples are pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and the 5' and N^4 acylated or alkylated derivatives of the active compound, as well as the 5'-monophosphate, diphosphate, or triphosphate derivatives or stablized phophate

prodrugs (alternatively referred to as "physiologically or pharmaceutically acceptable derivatives") or phosphate lipid prodrugs, as described herein.

Modifications of the active compounds, specifically at the N^4 and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

A preferred embodiment of the present invention is a method for the treatment of HIV infections in humans or other host animals, that includes administering an effective amount of one or more of an L-(2' or 3')-A-5-FddC nucleoside selected from the group consisting of, L-2'-A-5-FddC, and L-3'-A-5-FddC, or a physiologically acceptable prodrug thereof, including a phosphate, 5' and or N^4 alkylated or acylated derivative, or a physiologically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier. The compounds of this invention either possess anti-HIV activity, or are metabolized to a compound or compounds that exhibit anti-HIV activity. In a preferred embodiment, the L-(2' or 3')-A-5-FddC nucleoside is administered substantially in the form of a single isomer, i.e., at least approximately 95% in the designated stereoconfiguration.

Combination or Alternation Therapy

It has been recognized that drug-resistant variants of HIV can emerge after prolonged treatment with an antiviral agent. Drug resistance most typically occurs by mutation of a gene that encodes for an enzyme used in the viral life cycle. Recently, it has been demonstrated that the efficacy of a drug against HIV infection can be prolonged, augmented, or restored by administering the compound in combination or alternation with a second, and perhaps third, antiviral compound that induces a different mutation from that caused by the principle drug. Alternatively, the pharmacokinetics, biodistribution, or other parameter of the drug can be altered by such combination or alternation therapy. In general, combination therapy is typically preferred over alternation therapy because it induces multiple simultaneous stresses on the virus.

The second antiviral agent for the treatment of HIV, in one embodiment, can be a reverse transcriptase inhibitor (a "RTI"), which can be either a synthetic nucleoside (a "NRTI") or a non-nucleoside compound (a "NNRTI"). In an alternative embodiment, in the case of HIV, the second (or third) antiviral agent can be a protease inhibitor. In other embodiments, the second (or third) compound can be a pyrophosphate analog, or a fusion

binding inhibitor. A list compiling resistance data collected *in vitro* and *in vivo* for a number of antiviral compounds is found in Schinazi, et al, Mutations in retroviral genes associated with drug resistance, *International Antiviral News,* Volume 1(4), International Medical Press 1996.

Preferred examples of antiviral agents that can be used in combination or alternation with the compounds disclosed herein for HIV therapy include 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (FTC); the (-)-enantiomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (3TC); carbovir, acyclovir, interferon, AZT, DDI, DDC, D4T, CS-92 (3'-azido-2',3'-dideoxy-5-methyl-cytidine), and β-D-dioxolane nucleosides such as β-D-dioxolanyl-guanine (DXG), β-D-dioxolanyl-2,6-diaminopurine (DAPD), and β-D-dioxolanyl-6-chloropurine (ACP), MKC-442 (6-benzyl-1-(ethoxymethyl)-5-isopropyl uracil.

Preferred protease inhibitors include crixovan (Merck), nelfinavir (Agouron), ritonavir (Abbot), saquinavir (Roche), and DMP-450 (DuPont Merck).

Nonlimiting examples of compounds that can be administered in combination or alternation with any of the β-L-(2' or 3'-azido)-2',3'-dideoxy-5-fluorocytosines of the present invention include (1S,4R)-4-[2-amino-6-cyclopropyl-amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate ("1592", a carbovir analog; GlaxoWellcome); 3TC: (-)-ß-L-2',3'-dideoxy-3'-thiacytidine (GlaxoWellcome); a-APA R18893: a-nitro-anilino-phenylacetamide; A-77003; C2 symmetry-based protease inhibitor (Abbott); A-75925: C2 symmetry-based protease inhibitor (Abbott); AAP-BHAP: bisheteroarylpiperazine analog (Upjohn); ABT-538: C2 symmetry-based protease inhibitor (Abbott); AzddU: 3'-azido-2',3'-dideoxyuridine; AZT: 3'-azido-3'-deoxythymidine (GlaxoWellcome); AZT-p-ddI: 3'-azido-3'-deoxythymidilyl-(5',5')-2',3'-dideoxyinosinic acid (Ivax); BHAP: bisheteroarylpiperazine; BILA 1906: N-{1S-[[[3-[2S-{(1,1-dimethylethyl)amino]carbonyl}-4R-]3-pyridinylmethyl)thio]-1 -piperidinyl]-2R-hydroxy-1S-(phenylmethyl)-propyl]amino]carbonyl]-2-methylpropyl }-2-quinolinecarboxamide (Bio Mega/Boehringer-Ingelheim); BILA 2185: N-(1,1-dimethylethyl)-1-[2S-[[2-2,6-dimethyphenoxy)-1-oxoethyl]amino]-2R-hydroxy -4-phenylbutyl]4R-pyridinylthio)-2-piperidinecarboxamide (Bio Mega/Boehringer-Ingelheim); BM+51.0836: thiazolo-isoindolinone derivative; BMS 186,318: aminodiol derivative HIV-1 protease inhibitor (Bristol-Myers-Squibb); d4API: 9-[2,5-dihydro-5-(phosphonomethoxy)-2-furanel]adenine (Gilead); d4C: 2',3'-didehydro-2',3'-dideoxycytidine; d4T: 2',3'-didehydro-3'-deoxythymidine (Bristol-Myers-Squibb);

ddC; 2',3'-dideoxycytidine (Roche); ddI: 2',3'-dideoxyinosine (Bristol-Myers-Squibb); DMP-266: a 1,4-dihydro-2H-3, 1-benzoxazin-2-one; DMP-450: {[4R-(4-a,5-a,6-b,7-b)]-hexahydro-5,6-bis(hydroxy)-1,3-bis(3-amino)phenyl]methyl)-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one}-bismesylate (Avid); DXG:(-)-ß-D-dioxolane-guanosine (Triangle); EBU-dM:5-ethyl-1-ethoxymethyl-6-(3,5-dimethylbenzyl)uracil; E-EBU: 5-ethyl-1-ethoxymethyl-6-benzyluracil; DS: dextran sulfate; E-EPSeU: 1-(ethoxymethyl)-(6-phenylselenyl)-5-ethyluracil; E-EPU: 1-(ethoxymethyl)-(6-phenyl-thio)-5-ethyluracil; FTC: ß-2',3'-dideoxy-5-fluoro-3'-thiacytidine (Triangle); HBY097: S-4-isopropoxycarbonyl-6-methoxy-3-(methylthio-methyl)-3,4-dihydroquinoxalin -2(1H)-thione; HEPT: 1-[(2-hydroxyethoxy)methyl]6-(phenylthio)thymine; HIV-1:human immunodeficiency virus type 1; JM2763: 1,1'-(1,3-propanediyl)-bis-1,4,8,11-tetraazacyclotetradecane (Johnson Matthey); JM3100:1,1'-[1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane(Johnson Matthey); KNI-272: (2S,3S)-3-amino-2-hydroxy-4-phenylbutyric acid-containing tripeptide; L-697,593; 5-ethyl-6-methyl-3-(2-phthalimido-ethyl)pyridin-2(1H)-one; L-735,524: hydroxy-aminopentane amide HIV-1 protease inhibitor (Merck); L-697,661: 3-{[(-4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin -2(1 H)-one; L-FDDC: (-)-ß-L-5-fluoro-2',3'-dideoxycytidine; L-FDOC:(-)-ß-L-5-fluoro-dioxolane cytosine; MKC442: 6-benzyl-1-ethoxymethyl-5-isopropyluracil (I-EBU; Triangle/Mitsubishi); Nevirapine:11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyridol[3,2-b:2',3'-e]diazepin-6-one (Boehringer-Ingelheim); NSC648400: 1-benzyloxymethyl-5-ethyl-6-(alpha-pyridylthio)uracil (E-BPTU); P9941: [2-pyridylacetyl-IlePheAla-y(CHOH)]2 (Dupont Merck); PFA: phosphonoformate (foscarnet; Astra); PMEA: 9-(2-phosphonylmethoxyethyl)adenine (Gilead); PMPA: (R)-9-(2-phosphonyl-methoxypropyl)adenine (Gilead); Ro 31-8959: hydroxyethylamine derivative HIV-1 protease inhibitor (Roche); RPI-312: peptidyl protease inhibitor, 1-[(3s)-3-(n-alpha-benzyloxycarbonyl)-l-asparginyl)-amino-2-hydroxy-4-phenylbutyryl]-n-tert-butyl-l-proline amide; 2720: 6-chloro-3,3-dimethyl-4-(isopropenyloxycarbonyl)-3,4-dihydro-quinoxalin-2(1H)thione; SC-52151: hydroxyethylurea isostere protease inhibitor (Searle); SC-55389A: hydroxyethyl-urea isostere protease inhibitor (Searle); TIBO R82150: (+)-(5S)-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-thione (Janssen); TIBO 82913: (+)-(5S)-4,5,6,7,-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1jk]-[1,4]benzo-diazepin-2(1H)-thione (Janssen); TSAO-m3T:[2',5'-

bis-O-(tert-butyldimethylsilyl)-3'-spiro-5'-(4'-amino-1',2'-oxathiole-2',2'-dioxide)]-b-D-pentofuranosyl-N3-methylthymine; U90152: 1-[3-[(1-methylethyl)-amino]-2-pyridinyl]-4-[[5-[(methylsulphonyl)-amino]-lH -indol-2yl]carbonyl]piperazine; UC: thiocarboxanilide derivatives (Uniroyal); UC-781 =N-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-furancarbothioamide; UC -82 =N-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-thiophenecarbothioamide; VB 11,328: hydroxyethyl-sulphonamide protease inhibitor (Vertex); VX-478: hydroxyethylsulphonamide protease inhibitor (Vertex); XM 323: cyclic urea protease inhibitor (Dupont Merck) or DMP-266 (efavirenz, Sustiva).

Preparation of the Active Compounds

Stereochemistry

Since the 1' and 4' carbons of the sugar or dioxolanyl moiety (referred to below generically as the sugar moiety) of the nucleosides are chiral, their nonhydrogen substituents (CH_2OR and the pyrimidine or purine base, respectively) can be either cis (on the same side) or trans (on opposite sides) with respect to the sugar ring system. The four optical isomers therefore are represented by the following configurations (when orienting the sugar moiety in a horizontal plane such that the "primary" oxygen (that between the C1' and C4'-atoms is in back): "β" or "cis" (with both groups "up", which corresponds to the configuration of naturally occurring nucleosides, i.e., the D configuration), "β" or cis (with both groups "down", which is a nonnaturally occurring configuration, i.e., the L configuration), "α "or "trans" (with the C2 substituent "up" and the C5 substituent "down"), and trans (with the C2 substituent "down" and the C5 substituent "up").

The active nucleosides of the present invention are in the β-L-configuration, with the azido group in the ribosyl configuration.

Nucleotide Prodrugs

Any of the nucleosides described herein can be administered as a stabilized nucleotide prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the nucleoside. A number of nucleotide prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphate of the nucleoside will increase the stability of the nucleotide. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, **27** (1995) 1-17. Any of these can be used in combination with the disclosed nucleosides to achieve a desired effect.

In one embodiment, the L-(2' or 3')-A-5-FddC nucleoside is provided as 5'-hydroxyl lipophilic prodrug, i.e., a 5'-ether lipid or a 5'-phosphoether lipid. Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the nucleoside, preferably at the 5'-OH position of the nucleoside or lipophilic preparations, include U.S. Patent Nos. 5,149,794 (Sep. 22, 1992, Yatvin et al.); 5,194,654 (Mar. 16, 1993, Hostetler et al., 5,223,263 (June 29, 1993, Hostetler et al.); 5,256,641 (Oct. 26, 1993, Yatvin et al.); 5,411,947 (May 2, 1995, Hostetler et al.); 5,463,092 (Oct. 31, 1995, Hostetler et al.); 5,543,389 (Aug. 6, 1996, Yatvin et al.); 5,543,390 (Aug. 6, 1996, Yatvin et al.); 5,543,391 (Aug. 6, 1996, Yatvin et al.); and 5,554,728 (Sep. 10, 1996; Basava et al.).

Foreign patent applications that disclose lipophilic substituents that can be attached to the L-(2' or 3')-A-5-FddC nucleoside derivative of the present invention, or lipophilic preparations, include WO 89/02733, W0 90/00555, W0 91/16920, W0 91/18914, W0 93/00910, W0 94/26273, W0 96/15132, EP 0 350 287, EP 93917054.4, and W0 91/19721.

Additional nonlimiting examples of L-(2' or 3')-A-5-FddC nucleosides are those that contain substituents as described in the following publications. These derivatized nucleosides can be used for the indications described in the text or otherwise as antiviral agents, including as anti-HIV agents. Ho, D.H.W. (1973) Distribution of Kinase and deaminase of 1b-D-arabinofuranosylcytosine in tissues of man and mouse. *Cancer Res.* **33**, 2816-2820; Holy, A. (1993) Isopolar phosphorous-modified nucleotide analogues. In: De Clercq (Ed.), Advances in Antiviral Drug Design, Vol. I, JAI Press, pp. 179-231; Hong, C.I., Nechaev, A., and West, C.R. (1979a) Synthesis and antitumor activity of 1b-D-arabinofuranosylcytosine conjugates of cortisol and cortisone. *Biochem. Biophys. Rs. Commun.* 88, 1223-1229; Hong, C.I.,

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A general process for the stereospecific synthesis of 3'-substituted β-L-dideoxynucleosides is shown in Figure 1. A general process for the stereospecific synthesis of 2'-substituted β-L-dideoxynucleosides is shown in Figure 2. A detailed synthesis of β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine is provided in Figure 3 and in Example 1 below. A detailed synthesis of β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine is provided in Figure 4 and in Example 2 below.

Example 1 Preparation of β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine

Melting points were determined in open capillary tubes on a Gallenkamp MFB-595-010 M apparatus and are uncorrected. The UV absorption spectra were recorded on an Uvikon 931 (KONTRON) spectrophotometer in ethanol. ^{1}H-NMR spectra were run at room temperature in DMSO-d_6 with a Bruker AC 250 or 400 spectrometer. Chemical shifts are given in ppm, DMSO-d_5 being set at 2.49 ppm as reference. Deuterium exchange, decoupling experiments or 2D-COSY were performed in order to confirm proton assignments. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of

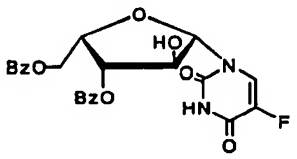
doublets), t (triplet), q (quadruplet), br (broad), m (multiplet). All J-values are in Hz. FAB mass spectra were recorded in the positive- (FAB>0) or negative (FAB<0) ion mode on a JEOL DX 300 mass spectrometer. The matrix was 3-nitrobenzyl alcohol (NBA) or a mixture (50:50, v/v) of glycerol and thioglycerol (GT). Specific rotations were measured on a Perkin-Elmer 241 spectropolarimeter (path length 1 cm) and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analysis were carried out by the "Service de Microanalyses du CNRS, Division de Vernaison" (France). Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values. Thin layer chromatography was performed on precoated aluminium sheets of Silica Gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorbency followed by charring with 10% ethanolic sulfuric acid and heating. Column chromatography was carried out on Silica Gel 60 (Merck, Art. 9385) at atmospheric pressure.

1-(2-O-Acetyl-3,5-di-O-Benzoyl-β-L-Xylofuranosyl)-5-Fluorouracil (2)

A suspension of 5-fluorouracil (5.0 g, 38.4 mmol) was treated with hexamethyldisilazane (HMDS, 260 mL) and a catalytic amount of ammonium sulfate during 18 h under reflux. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the residue obtained as a colorless oil was diluted with anhydrous 1,2-dichloroethane (260 mL). To the resulting solution was added 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-L-xylofuranose 1 (11.3 g, 25.6 mmol) [Ref.: Gosselin, G.; Bergogne, M.-C.; Imbach, J.-L., "Synthesis and Antiviral Evaluation of β-L-Xylofuranosyl Nucleosides of the Five Naturally Occurring Nucleic Acid Bases", *Journal of Heterocyclic Chemistry,* 1993, **30** (Oct.-Nov.), 1229-1233] in anhydrous 1,2-dichloroethane (130 mL), followed by addition of trimethylsilyl trifluoromethanesulfonate (TMSTf, 9.3 mL, 51.15 mmol). The solution was stirred for 6 h at room temperature under argon atmosphere, then diluted with chloroform (1 L), washed with the same volume of a saturated aqueous sodium hydrogen carbonate solution and finally with water (2× 800 mL). The organic phase was dried over sodium sulphate, then evaporated under reduced pressure. The resulting crude material was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-4%) in methylene chloride] to give 2 (11.0 g, 84% yield) as a white foam; mp = 96-98°C; UV (ethanol): λ_{max} = 228 nm (ε = 25900) 266 nm (ε = 9000), λ_{min} = 250 nm (ε = 7200); ^{1}H-NMR (DMSO-d_6): δ 11.1 (br s, 1H, NH), 8.05 (1H, H-6, $J_{6\text{-}F5}$ = 6.8 Hz), 7.9-7.4 (m, 10H, 2 C_6H_5CO), 5.99 (d, 1H,

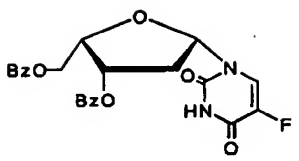
H-1', $J_{1'-2'}$ = 3.1 Hz), 5.74 (dd, 1H, H-3', $J_{3'-2'}$ = 4.2 Hz and $J_{3'-4'}$ = 2.3 Hz), 5.54 (t, 1H, H-2', $J_{2'-1'} = J_{2'-3'}$ = 2,9 Hz), 4.8-4.6 (m, 3H, H-4', H-5' and H-5"); MS: FAB>0 (matrix GT) *m/z* 513 $(M+H)^+$, 383 $(S)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 511 $(M-H)^-$, 469 $(M-CH_3CO)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -91 (c, 0.88 DMSO); Anal $C_{25}H_{21}FN_2O_9$ (C, H, N, F).

1-(3,5-Di-O-benzoyl-β-L-xylofuranosyl)-5-fluorouracil 3



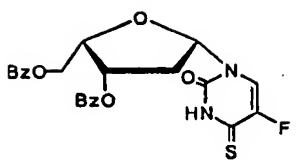
Hydrazine hydrate (2.80 mL, 57.4 mmol) was added to a solution of 1-(2-*O*-acetyl-3,5-di-*O*-benzoyl-β-L-xylofuranosyl)-5-fluorouracil 2 (9.80 g, 19.1 mmol) in acetic acid (35 mL) and pyridine (150 mL). The resulting solution was stirred overnight at room temperature. Acetone (50 mL) was added and the mixture was stirred during 2 h. The reaction mixture was concentrated to a small volume and partitioned between ethyl acetate (200 mL) and water (200 mL). Layers were separated and the organic phase was washed with a saturated aqueous sodium hydrogen carbonate solution (2× 200 mL), and finally with water (2× 200 mL). The organic phase was dried over sodium sulphate, then evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-5%) in methylene chloride] to give pure 3 (7.82 g, 87%), which was crystallized from methylene chloride; mp = 93-97°C; UV (ethanol): λ_{max} = 227 nm (ε = 22800) 267 nm (ε = 8200), λ_{min} = 249 nm (ε =5900); ^{1}H-NMR (DMSO-d_6): δ 11.9 (br s, 1H, NH), 8.06 (d, 1H, H-6, J_{6-F5} = 6.9 Hz), 8.0-7.4 (m, 10H, 2 C_6H_5CO), 6.35 (d, 1H, OH-2', $J_{OH-2'}$ = 3.8 Hz), 5.77 (d, 1H, H-1', $J_{1'-2'}$ = 3.3 Hz), 5.43 (dd, 1H, H-3', $J_{3'-2'}$ = 3.1 Hz and $J_{3'-4'}$ = 1.9 Hz) 4.8-4.6 (m, 3H, H-4', H-5' and H-5"), 4.43 (t, 1H, H-2', J = 2.3 Hz); MS: FAB>0 (matrix GT) *m/z* 941 $(2M+H)^+$, 471 $(M+H)^+$, 341 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 939 $(2M-H)^-$, 469 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -110 (c, 1.55 DMSO).

1-(2-Deoxy-3,5-di-O-benzoyl-β-L-*threo*-pentofuranosyl)-5-fluorouracil 5



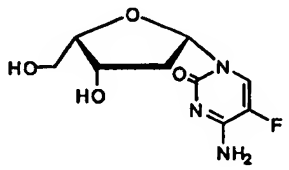
To a solution of 1-(3,5-di-*O*-benzoyl-β-L-xylofuranosyl)-5-fluorouracil 3 (15.4 g, 32.7 mmol) in anhydrous acetonitrile (650 mL) were added *O*-phenyl chlorothionoformate (6.80 mL, 49.1 mmol) and 4-dimethylaminopyridine (DMAP, 12.0 g, 98.2 mmol). The resulting solution was stirred at room temperature under argon during 1 h and then evaporated under reduced pressure. The residue was dissolved in methylene chloride (350 mL) and the organic solution was successively washed with water (2× 250 mL), with an ice-cold 0.5 N hydrochloric acid (250 mL) and with water (2× 250 mL), dried over sodium sulphate and evaporated under reduced pressure. The crude material 4 was co-evaporated several times with anhydrous dioxane and dissolved in this solvent (265 mL). To the resulting solution were added under argon tris(trimethylsilyl)silane hydride (12,1 mL, 39.3 mmol) and α,α'-azoisobutyronitrile (AIBN, 1.74 g, 10.8 mmol). The reaction mixture was heated and stirred at 100°C for 2.5 h under argon, then cooled to room temperature and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-2%) in chloroform] to give pure 5 (13.0 g, 87%), which was crystallized from a diethyl ether/methanol mixture; mp = 182-184°C; UV (ethanol): λ_{max} = 229 nm (ε = 25800), 269 nm (ε = 9300), λ_{min} = 251 nm (ε = 6500); ^{1}H-NMR (DMSO-d_6): δ 11.8 (br s, 1H, NH), 8.05 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 8.0-7.4 (m, 10H, 2 C_6H_5CO), 6.15 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 7.4 Hz), 5.68 (t, 1H, H-3', $J_{3'\text{-}2'} = J_{3'\text{-}4'}$ = 4.2 Hz), 4.8-4.6 (m, 2H, H-5' and H"-5), 4.6 (m, 1H, H-4'), 3.0-2.8 (m, 1H, H-2'), 2.5-2.3 (d, 1H, H-2", J = 14.8 Hz); MS: FAB>0 (matrix GT) *m/z* 455 $(M+H)^+$, 325 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 452 $(M-H)^-$, 129 $(B)^-$; $[\alpha]_D^{20}$ = -125 (c 1.05 DMSO); Anal $C_{23}H_{19}FN_2O_7$ (C, H, N, F).

1-(2-Deoxy-3,5-di-o-benzoyl-β-L-threo-pentofuranosyl)-4-thio-5-fluorouracil 6



Lawesson's reagent (3.1 g, 7.70 mmol) was added under argon to a solution of 5 (5.0 g, 11.0 mmol) in anhydrous 1,2-dichloroethane (200 mL) and the reaction mixture was stirred overnight under reflux. The solvent was then evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-2%) in chloroform] to give the 4-thio intermediate 6 (80% yield).as a yellow foam; mp = 178-179°C; UV (ethanol): λ_{max} = 230 nm (ε = 24900), 273 nm (ε = 6900), 333 nm (ε = 19200), λ_{min} = 258 nm (ε = 5900), 289 nm (ε = 5300); ^{1}H-NMR (DMSO-d_6): δ 13.1 (br s, 1H, NH), 8.10 (d, 1H, H-6, $J_{6\text{-}F5}$ = 4,6 Hz), 8.1-7.4 (m, 10H, 2 C_6H_5CO), 6.09 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 7.3 Hz), 5.68 (t, 1H, H-3', $J_{3'\text{-}2'}$ = $J_{3'\text{-}4'}$ = 4.1 Hz), 4.9-4.8 (m, 2H, H-5' and H-5"), 4.7 (m, 1H, H-4'), 2.9 (m, 1H, H-2'), 2.5 (m, 1H, H-2"); MS: FAB>0 (matrix GT) *m/z* 941 $(2M+H)^+$, 471 $(M+H)^+$, 325 $(S)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 (matrix GT) *m/z* 469 $(M-H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -271 (c, 0,90 DMSO); Anal $C_{23}H_{19}FN_2O_6S$ (C, H, N, F).

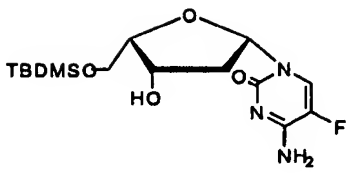
1-(2-Deoxy-β-L-*threo*-pento furanosyl)-5-fluorocytosine 7



A solution of this 4-thio intermediate 6 (1.0 g, 2.13 mmol) in methanolic ammonia (previously saturated at -10°C and tightly stopped) (60 mL) was heated at 100°C in a stainless-steel bomb for 3 h and then cooled to 0°C. The solution was evaporated to dryness under reduced pressure and the residue co-evaporated several times with methanol. The crude material was dissolved in water and the resulting solution was washed four times with methylene chloride. The aqueous layer was evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of

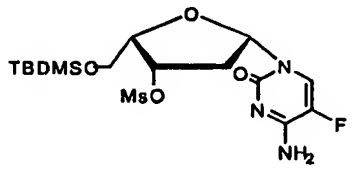
methanol (3-20%) in methylene chloride]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0,45 μm, Millipore) to provide 0.44 g of 7 (84% yield) which was crystallized from an ethyl acetate/methanol mixture; mp = 199-201°C; UV (ethanol): λ_{max} = 226 nm (ε = 7700), 281 nm (ε = 8500), λ_{min} = 262 nm (ε = 6300); ^{1}H-NMR (DMSO-d_6): δ 7.99 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.4 Hz), 7.7-7.4 (br d, 2H, NH_2), 5.98 (d, 1H, H-1', $J_{1'\text{-}2'}$ = 8.1 Hz), 5.25 (d, 1H, OH-3', $J_{OH\text{-}3'}$ = 3.4 Hz), 4.71 (t, 1H, OH-5', $J_{OH\text{-}5'} = J_{OH\text{-}5''}$ = 5.6 Hz), 4.2 (m, 1H, H-3'), 3.8-3.6 (m, 3H, H-4', H-5' and H-5"), 2.5 (m, 1H, H-2'), 1.8 (m, 1H, H-2"); MS: FAB>0 (matrix GT) *m/z* 491 $(2M+H)^+$, 246 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 (matrix GT) *m/z* 489 $(2M-H)^-$, 244 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -21 (c, 0.92 DMSO); Anal $C_9H_{12}FN_3O_4$ (C, H, N, F).

1-(2-Deoxy-5-O-*t*-butyldimethyl silyl-β-L-*threo*-pentofuranosyl)-5-fluorocytosine 8



To a solution of 7 (1.69 g, 6.89 mmol) in dry pyridine (35 mL) was added dropwise under argon atmosphere *t*-butyldimethylsilyl chloride (1.35 g, 8.96 mmol) and the mixture was stirred for 5 h at room temperature. Then the mixture was poured onto a saturated aqueous sodium hydrogen carbonate solution (100 mL) and extracted with chloroform (3× 150 mL). Combined extracts were washed with water (2× 200 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (2-10%) in methylene chloride] to give pure 8 (2.94 g, 87%), as a white solid: mp 177-179°C; UV (ethanol): λ_{max} 241 nm (ε 9900), 282 nm (ε 10000), λ_{min} 226 nm (ε 8200), 263 nm (ε 7600); ^{1}H NMR (DMSO-d_6): δ 7.95 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.3 Hz), 7.8-7.3 (br d, 2H, NH_2), 6.00 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 6.1 Hz and $J_{1'\text{-}2''}$ = 1.9 Hz), 5.3 (br s, 1H, OH-3'), 4.2 (br s, 1H, H-3'), 3.9-3.7 (m, 3H, H-4', H-5' and H-5"), 2.5 (m, 1H, H-2'), 1.81 (br d, 1H, H-2", J = 14.6 Hz), 0.86 (s, 9H, $(CH_3)_3$C-Si), 0.05 (s, 6H, $(CH_3)_2$Si); MS (matrix GT): FAB>0 *m/z* 719 $(2M+H)^+$, 360 $(M+H)^+$, 130 $(BH_2)^+$, 115 $(TBDMS)^+$; FAB<0 *m/z* 717 $(2M-H)^-$, 358 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -23 (c, 0.96 DMSO).

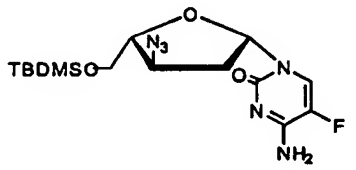
1-(2-Deoxy-3-O-mesyl-5-O-*t*-butyl dimethylsilyl-β-L-*threo*-pento furanosyl)-5-fluorocytosine 9



A suspension of 8 (0.70 g, 1.96 mmol) in dry pyridine (30 mL) was stirred under argon and cooled to 0°C. Methanesulfonyl chloride (MsCl, 0.46 mL, 5.88 mmol) was added dropwise and the reaction mixture stirred at 0°C for 5 h. Then the mixture was poured onto

ice/water (100 mL) and extracted with chloroform (3× 100 mL). Combined extracts were washed with a 5% aqueous sodium hydrogen carbonate solution (100 mL), with water (2× 100 mL), dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (8-12%) in toluene] to give pure 9 (0.56 g, 65%) as a white solid: mp 83-84 °C; UV (ethanol): λ_{max} 242 nm (ε 8500), 282 nm (ε 8800), λ_{min} 225 nm (ε 6400), 264 nm (ε 6300); ^{1}H NMR (DMSO-d_6): δ 7.8-7.3 (br d, 2H, NH_2), 7.60 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 5.93 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 4.5 Hz and $J_{1'\text{-}2''}$ = 2.0 Hz), 5.2 (m, 1H, H-3'), 4.1 (m, 1H, H-4'), 3.9-3.7 (m, 2H, H-5' and H-5"), 3.17 (s, 3H, CH_3SO_2), 2.7 (m, 1H, H-2'), 2.1 (m, 1H, H-2"), 0.99 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 875 $(2M+H)^+$, 438 $(M+H)^+$, 342 $(M-CH_3SO_3)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 873 $(2M-H)^-$, 436 $(M-H)^-$, 128 $(B)^-$, 95 $(CH_3SO_3)^-$; $[\alpha]_D^{20}$ = -28 (c, 0.96 DMSO).

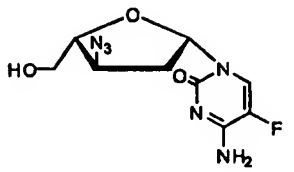
1-(2,3-Dideoxy-3-azido-5-O-*t*-butyl dimethylsilyl-β-L-*erythro*-pento furanosyl)-5-fluorocytosine 10



To a solution of 9 (520 mg, 1.19 mmol) in anhydrous dimethylformamide (12 mL) was added lithium azide moistened with 10% methanol (300 mg, 5.31 mmol). The reaction mixture was stirred at 100°C during 2.5 h, and then cooled to room temperature, poured onto ice/water (200 mL) and extracted with chloroform (3× 100 mL). Combined extracts were washed with saturated aqueous sodium hydrogen carbonate solution (2× 100 mL), with water (5× 100 mL), and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : methanol (4%) in chloroform] to give pure 10 (327 mg, 72%), which was crystallized from a diethyl ether/methanol mixture: mp 146-147°C; UV (ethanol): λ_{max} 243 nm (ε 8700), 283 nm (ε 8400), λ_{min} 226 nm (ε 7200), 264 nm (ε 6700) ; ^{1}H NMR (DMSO-d_6): δ 7.90 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 7.8-7.5 (br d, 2H, NH_2), 6.0 (m, 1H, H-1'), 4.3 (m, 1H, H-3'), 3.9-3.7 (m, 3H, H-4', H-5' and H"-5), 2.4-2.2 (m, 2H, H-2' and H-2"), 0.87 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H,

$(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 769 $(2M+H)^+$, 385 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 383 $(M-H)^-$; $[\alpha]_D^{20}$ = -67 (c, 0.96 DMSO).

1-(2,3-Dideoxy-3-azido-β-L-*erythro*-pentofuranosyl)-5-fluorocytosine 11 (3'-N_3-β-L-5-FddC)



A 1 M solution of tetrabutylammonium trifluoride in tetrahydrofurane (TBAF/THF, 1.53 mL, 1.53 mmol) was added to a solution of **10** (295 mg, 0.67 mmol) in anhydrous THF (4 mL). The resulting mixture was stirred at room temperature for 1.5 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (4-8%) in chloroform]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0,45 μm, Millipore) to give pure **11** (199 mg, 96%), which was crystallized from ethanol: mp 188-189°C (lit.: mp 164-166°C for the D-enantiomer); UV (ethanol): λmax 243 nm (ε 8700), 283 nm (ε 8100), λmin 226 nm (ε 7100), 264 nm (ε 6500) ; ^{1}H NMR (DMSO-d_6): δ 8.08 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.3 Hz), 7.8-7.5 (br d, 2H, NH_2), 6.0 (m, 1H, H-1'), 5.3 (br s, 1H, OH-5'), 4.4 (m, 1H, H-3'), 3.8 (m, 1H, H-4'), 3.7-3.5 (m, 2H, H-5' and H-5"), 2.3 (m, 2H, H-2' and H-2"); MS (matrix GT): FAB>0 *m/z* 811 $(3M+H)^+$, 725 $(2M+2G+H)^+$, 633 $(2M+G+H)^+$, 541 $(2M+H)^+$, 363 $(M+G+H)^+$, 271 $(M+H)^+$, 142 $(S)^+$, 130 $(BH_2)^+$; FAB<0*m/z* 647 $(2M+T-H)^-$, 539 $(2M-H)^-$, 377 $(M+T-H)^-$, 269 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -31 (c, 0.90 DMSO); Anal. ($C_9H_{11}FN_6O_3$) C, H, N, F.

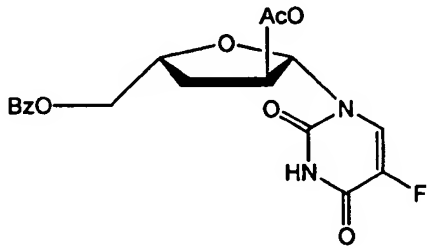
Analytical data

Compound	Formula	Anal Calculated				Anal Found			
		C	H	N	F	C	H	N	F
2	$C_{25}H_{21}FN_2O_9$	58.59	4.13	5.47	3.71	58.33	4.25	4.24	3.49
5	$C_{23}H_{19}FN_2O_7$	60.79	4.21	6.17	4.18	61.22	4.26	6.18	3.90
6	$C_{23}H_{19}FN_2O_6S$	58.71	4.07	5.96	4.04	58.25	4.10	5.91	4.00
7	$C_9H_{12}FN_3O_4$	44.08	4.87	17.17	7.75	43.87	5.13	16.81	7.42
11	$C_9H_{11}FN_6O_3$	40.00	4.10	31.10	7.03	40.35	3.83	31.38	7.12

Example 2 Preparation of β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine

General procedures and instrumentation used have been described in Example 1 in the Experimental protocols part of the synthesis of the 3' isomer (3'-N_3-β-L-FddC).

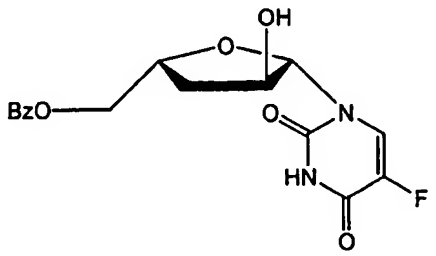
1-(2-O-acetyl-3-deoxy-5-O-benxoyl-β-L-erythro-pentofuranosyl)-5-fluorouracil 13



A suspension of 5-fluorouracil (5.15 g, 39.6 mmol) was treated with hexamethyldisilazane (HMDS, 257 mL) and a catalytic amount of ammonium sulfate during 18 h under reflux. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the residue obtained as a colourless oil was diluted with anhydrous 1,2-dichloroethane (290 mL). To the resulting solution was added 1,2-di-*O*-acetyl-3-deoxy-5-*O*-

benzoyl-L-*erythro*-pentofuranose 12 (8.5 g, 26.4 mmol) [Ref.: Mathé, C., *Ph.D. Dissertation*, Université de Montpellier II -Sciences et Techniques du Languedoc, Montpellier (France), September 13, 1994; Gosselin, G.; Mathé, C.; Bergogne, M.-C.; Aubertin, A.M.; Kirn, A.; Sommadossi, J.P.; Schinazi, R.F.; Imbach, J.L., "2'- and/or 3'-deoxy-β-L-pentofuranosyl nucleoside derivatives: stereospecific synthesis and antiviral activities," *Nucleosides & Nucleotides,* 1994, **14** (3-5), 611-617] in anhydrous 1,2-dichloroethane (120 mL), followed by addition of trimethylsilyl trifluoromethanesulfonate (TMSTf, 9.6 mL, 52.8 mmol). The solution was stirred for 5 h at room temperature under argon atmosphere, then diluted with chloroform (200 mL), washed with the same volume of a saturated aqueous sodium hydrogen carbonate solution and finally with water (2× 300 mL). The organic phase was dried over sodium sulphate, then evaporated under reduced pressure. The resulting crude material was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-6%) in methylene chloride] to give pure 13 (8.59 g, 83%), which was crystallized from toluene: mp 65-68°C; UV (ethanol): λ_{max} 228 nm (ε 11200) 268 nm (ε 14000), λ_{min} 242 nm (ε 7800); ^{1}H NMR (DMSO-d_6): δ 11.9 (br s, 1H, NH), 8.0-7.5 (m, 6H, C_6H_5CO and H-6), 5.8 (m, 1H, H-1'), 5.3 (m, 1H, H-2'), 4.6-4.5 (m, 3H, H-4', H-5' and H-5"), 2.4-2.3 (m, 1H, H-3'), 2.1-2.0 (m, 4H, H-3" and CH_3CO); MS (matrix GT): FAB>0 *m/z* 393 $(M+H)^+$, 263 $(S)^+$, 105 $(C_6H_5CO)^+$; FAB<0 *m/z* 391 $(M-H)^-$, 331 $(M-[CH_3CO_2H]-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -8 (*c*,1.00 DMSO); Anal. ($C_{18}H_{17}FN_2O_7$; $^2/_3$ C_7H_8) C, H, N, F.

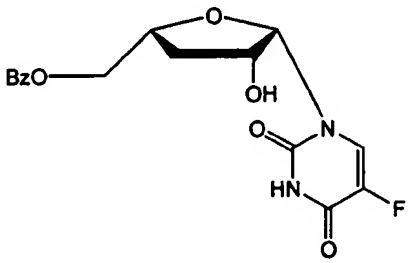
1-(3-Deoxy-5-O-benzoyl-β-L-erythro-pentofuranosyl)-5-fluorouracil 14



To a solution of 13 (5.90 g, 15.0 mmol) in tetrahydrofurane (THF, 175 mL), was added sodium methoxide (2.84 g, 52.6 mmol). The resulting suspension was stirred at room temperature during 5 h and then neutralized by addition of Dowex 50 W X 2 (H^+ form). The

resin was filtered and washed with warm methanol, and the combined filtrates were evaporated to dryness. Column chromatography of the residue on silica gel [eluent: stepwise gradient of methanol (0-8%) in methylene chloride] afforded 14 (4.11 g, 78%), which was crystallized from a methylene chloride/methanol mixture: mp 154-156°C; UV (ethanol): λ_{max} 226 nm (ε 23000), 268 nm (ε 16000), λ_{min} 246 nm (ε 8900); ^{1}H NMR (DMSO-d_6): δ 11.8 (br s, 1H, NH), 8.0-7.5 (m, 6H, C_6H_5CO and H-6), 5.6 (br s, 2H, H-1' and OH-2'), 4.5 (m, 3H, H-4', H-5' and H-5"), 4.3 (m, 1H, H-2'), 2.1-2.0 (m, 1H, H-3'), 1.9 (m, 1H, H-3"); MS (matrix GT): FAB>0 *m/z* 701 $(2M+H)^+$, 351 $(M+H)^+$, 221 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 *m/z* 1049 $(3M-H)^-$, 699 $(2M-H)^-$, 441 $(M+G-H)^-$, 349 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -3 (*c*, 1.04 DMSO); Anal. ($C_{16}H_{15}FN_2O_6$) C, H, N, F

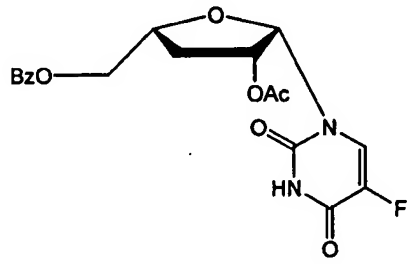
1-(3-Deoxy-5-O-benzoyl-β-L-threo-pentofuranosyl)-5-fluorouracil 15



Dicyclohexylcarbodiimide (DCC, 3.53 g, 17.1 mmol) and dichloroacetic acid (0.235 mL, 2.56 mmol) were added to a solution of 14 (2.00 g, 5.71 mmol) in anhydrous benzene (50 mL), DMSO (35 mL) and pyridine (0.46 mL). The resulting solution was stirred at room temperature under argon during 4 h and diluted with ethyl acetate (300 mL). Oxalic acid (1.54 g, 17.1 mmol) dissolved in methanol (4.6 mL) was added and the reaction mixture was stirred at room temperature during 1 h and then filtered to eliminate precipitated dicyclohexylurea (DCU). The filtrate was washed with brine (3x 300 mL), with a saturated aqueous sodium hydrogen carbonate solution (2□ 300 mL) and finally with water (3x 200 mL) before being dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was co-evaporated several times with absolute ethanol and dissolved in a mixture of absolute ethanol (31 mL) and anhydrous benzene (15 mL). The resulting solution was then cooled to 0°C and sodium borohydride ($NaBH_4$, 0.32 g, 8.56 mmol) was added. The

reaction mixture was stirred at room temperature under argon during 1 h and diluted with ethyl acetate (300 mL) filtered. The filtrate was washed with a saturated aqueous sodium chloride solution (3x 300 mL) and with water (2□ 200 mL) before being dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-6%) in chloroform] to give pure 15 (1.10 g, 55%), as a white foam: mp 171-172□C; UV (ethanol): λ_{max} 228 nm (ε 14700) 270 nm (ε 9100), λ_{min} 248 nm (ε 5000); ^{1}H NMR (DMSO-d_6): δ 11.8 (br s, 1H, NH), 8.0-7.5 (m, 6H, C_6H_5CO and H-6), 5.90 (dd, 1H, H-1', $J_{1'-2'}$ = 4.1 Hz and $J_{1'-F5}$ = 1.8 Hz), 5,5 (br s, 1H, OH-2'), 4.7 (br q, 1H, H-4', J = 11.7 Hz and J = 7.0 Hz), 4.4-4.3 (m, 3H, H-2', H-5' and H-5"), 2.4 (m, 1H, H-3'), 1.9-1.8 (m, 1H, H-3"); MS (matrix GT): FAB>0 *m/z* 701 $(2M+H)^+$, 351 $(M+H)^+$, 221 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB<0 *m/z* 1049 $(3M-H)^-$, 699 $(2M-H)^-$, 349 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$; $[\alpha]_D^{20}$ = -101 (*c*, 0.70 DMSO).

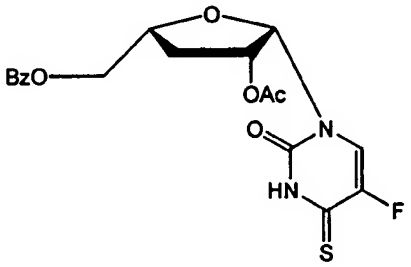
1-(2-O-acetyl-3-deoxy-5-O-benzoyl-β-L-threo-pentofuranosyl)-5-fluorouracil 16



Acetic anhydride (0.88 mL, 9.28 mmol) was added under argon to a solution of 15 (2.50 g, 7.14 mmol) in dry pyridine (50 mL) and the resulting mixture was stirred at room temperature for 22 h. Then, ethanol was added and the solvents were evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-2%) in methylene chloride] to give pure **16** (2.69 g, 96%) as a white foam; mp = 68-70°C (foam); UV (ethanol) : λ_{max}= 239 nm (ε= 15000) 267 nm (ε= 8800), λ_{min}= 248 nm (ε= 5600) ; ^{1}H NMR (DMSO-d_6) : δ ppm 11.9 (br s, 1H, NH), 8.1-7.5 (m, 6H, C_6H_5CO and H-6), 6.10 (d, 1H, H-1', $J_{1'-2'}$= 4.3 Hz), 5.4 (m, 1H, H-2'), 4.6-4.4 (m, 3H, H-4', H-5' and H-5"), 2.6 (m, 1H, H-3'), 2.03 (m, 1H, H-3"), 1,86 (s, 3H, CH_3CO) ; MS (matrix GT): FAB>0 *m/z* 785 $(2M+H)^+$, 393 $(M+H)^+$, 263 $(S)^+$, 131 $(BH_2)^+$, 105 $(C_6H_5CO)^+$,

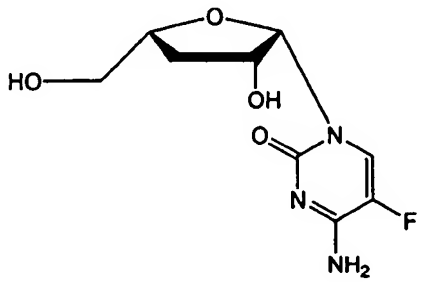
43 $(CH_3CO)^+$; FAB<0 *m/z* 391 $(M-H)^-$, 129 $(B)^-$, 121 $(C_6H_5CO_2)^-$, 59 $(CH_3CO_2)^-$; $[\alpha]_D^{20}$= -81 (*c*, 0.95 DMSO).

1-(2-O-acetyl-3-deoxy-5-O-benzoyl-β-L-threo-pentofuranosyl)-4-thio-5-fluorouracil 17



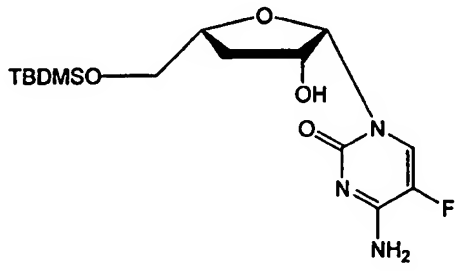
Lawesson's reagent (1.9 g, 4.69 mmol) was added under argon to a solution of 16 (2.63 g, 6.70 mmol) in anhydrous 1,2-dichloroethane (165 mL) and the reaction mixture was stirred overnight under reflux. The solvent was then evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (0-3%) in methylene chloride] to give the 4-thio derivative 17 (2.65 g, 96% yield) as a yellow foam; mp = 78-79°C (foam) ; UV (ethanol) : λ_{max}= 230 nm (ε= 15900) 334 nm (ε= 15600), λ_{min}= 288 nm (ε= 3200) ; ^{1}H NMR (DMSO-d_6) : δ ppm 13.2 (br s, 1H, NH), 8.1-7.5 (m, 6H, C_6H_5CO and H-6), 6.08 (d, 1H, H-1', $J_{1'-2'}$= 4.3 Hz), 5.4 (m, 1H, H-2'), 4.7-4.4 (m, 3H, H-4', H-5' and H-5"), 2.6 (m, 1H, H-3'), 2.0 (m, 1H, H-3"), 1.84 (s, 3H, CH_3CO) ; MS (matrix GT): FAB>0 *m/z* 409 $(M+H)^+$, 263 $(S)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$, 43 $(CH_3CO)^+$; FAB<0 *m/z* 407 $(M-H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$, 59 $(CH_3CO_2)^-$; $[\alpha]_D^{20}$= -155 (*c*, 1.00 DMSO).

1-(3-Deoxy-β-L-threo-pentofuranosyl)-5-fluorocytosine 18



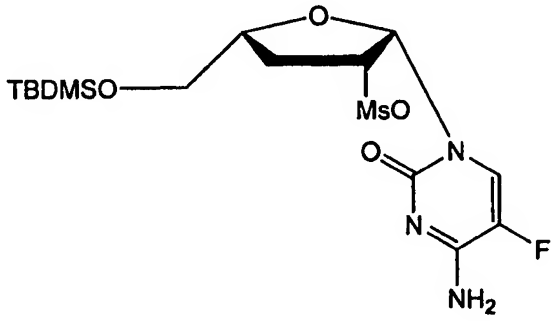
A solution of the 4-thio derivative 17 (0.86 g, 2.19 mmol) in methanolic ammonia (previously saturated at -10°C and tightly stopped) (44 mL) was heated at 100°C in a stainless-steel bomb for 3 h and then cooled to 0°C. The solution was evaporated to dryness under reduced pressure and the residue co-evaporated several times with methanol. The crude material was dissolved in water and the resulting solution was washed four times with methylene chloride. The aqueous layer was evaporated under reduced pressure and the residue was purified by silica gel column chromatography [eluent: stepwise gradient of methanol (3-12%) in chloroform]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0.45 µm, Millipore) to provide 0.46 g of **18** (86% yield) which was crystallized from a methylene/methanol mixture; mp = 137-138°C ; UV (ethanol) : λ_{max}= 240 nm (ε= 8300) 284 nm (ε= 8100), λ_{min}= 226 nm (ε= 7300) 263 nm (ε= 5500) ; ^{1}H NMR (DMSO-d_6) : δ ppm 8.34 (d, 1H, H-6, $J_{6\text{-}F5}$= 7.5 Hz), 7.7-7.4 (br pd, 2H, NH_2), 5.83 (dd, 1H, H-1', $J_{1'\text{-}2'}$= 4.4 Hz, $J_{1'\text{-}F5}$= 1.9 Hz), 5.22 (d, 1H, OH-2', $J_{OH\text{-}2'}$= 5.1 Hz), 5.15 (t, 1H, OH-5', $J_{OH\text{-}5'}$= $J_{OH\text{-}5''}$= 4.8 Hz), 4.3 (m, 1H, H-2'), 4.0 (m, 1H, H-4'), 3.6-3.5 (m, 2H, H-5' and H-5") 2.2 (m, 1H, H-3'), 1.7 (m, 1H, H-3") ; MS (matrix GT): FAB>0 *m/z* 491 $(2M+H)^+$, 246 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 244 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$= -135 (*c*, 0.89 DMSO). Elemental analysis, $C_9H_{12}FN_3O_4$, ½ H_2O; Calc. C= 42.52 ; H= 5.15 ; N= 16.53 ; F= 7.47; Found: C= 43.16 ; H= 5.32 ; N= 16.97 ; F= 6.92.

1-(3-Deoxy-5-O-t-butyldimethylsilyl-β-L-threo-pentofuranosyl)-5-fluorocytosine 19



To a solution of **18** (1.38 g, 5.63 mmol) in dry pyridine (30 mL) was added dropwise under argon atmosphere *t*-butyldimethylsilyl chloride (1.10 g, 7.32 mmol) and the mixture was stirred for 10 h at room temperature. Then the mixture was poured onto a saturated aqueous sodium hydrogen carbonate solution (100 mL) and extracted with chloroform (3× 150 mL). Combined extracts were washed with water (2× 200 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (2-10%) in methylene chloride] to give pure **19** (1.74 g, 86% yield) as a white solid: mp 202-204°C; UV (ethanol): λ_{max} 241 nm (ε 7800), 284 nm (ε 7800), λ_{min} 226 nm (ε 6600), 263 nm (ε 5400) ; ^{1}H NMR (DMSO-d_6): δ 7.77 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.1 Hz), 7.7-7.3 (br d, 2H, NH_2), 6.88 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 4.9 Hz and $J_{1'\text{-}F5}$ = 1.9 Hz), 5.24 (d, 1H, OH-3', $J_{OH\text{-}3'}$ = 4.6 Hz), 4.4 (m, 1H, H-2'), 4.0 (m, 1H, H-4'), 3.8-3.7 (m, 2H, H-5' and H-5"), 2.2 (m, 1H, H-3'), 1.7 (m, 1H, H-3"), 0.84 (s, 9H, $(CH_3)_3C$-Si), 0.06 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 1437 $(4M+H)^+$, 1078 $(3M+H)^+$, 719 $(2M+H)^+$, 360 $(M+H)^+$, 231 $(S)^+$, 130 $(BH_2)^+$, 115 $(TBDMS)^+$; FAB<0 *m/z* 1076 $(3M-H)^-$, 717 $(2M-H)^-$, 358 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = -107 (*c*, 0.88 DMSO).

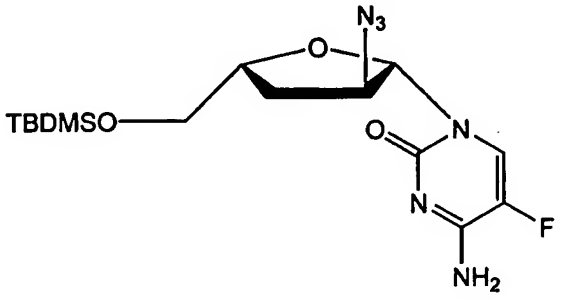
1-(3-Deoxy-2-O-mesyl-5-O-*t*-butyl dimethylsilyl-β-L-*threo*-pentofuranosyl)-5-fluorocytosine 20



A suspension of **19** (1.70 g, 4.73 mmol) in dry pyridine (80 mL) was stirred under argon and cooled to 0°C. Methanesulfonyl chloride (MsCl, 1.21 mL, 15.6 mmol) was added dropwise and the reaction mixture stirred at 0°C for 5 h. Then the mixture was poured onto ice/water (300 mL) and extracted with chloroform (3× 300 mL). Combined extracts were washed with a 5% aqueous sodium hydrogen carbonate solution (300 mL), with water (2×

300 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (8-12%) in toluene] to give pure **20** (1.41 g, 68% yield) as a white solid: mp 75-76 °C; UV (ethanol): λ_{max} 243 nm (ε 8100), 282 nm (ε 7300), λ_{min} 225 nm (ε 6000), 265 nm (ε 6000); ^{1}H NMR (DMSO-d_6): δ 7.9-7.6 (br d, 2H, NH_2), 7.85 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 6.08 (dd, 1H, H-1', $J_{1'\text{-}2'}$ = 5.2 Hz and $J_{1'\text{-}F5}$ = 1.6 Hz), 5.4 (m, 1H, H-2'), 4.1 (m, 1H, H-4'), 3.9 (m, 1H, H-5'), 3.7 (m, 1H, H-5"), 3.11 (s, 3H, CH_3SO_2), 2.47 (m, 1H, H-3'), 2.0 (m, 1H, H-2"), 0.85 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 1312 $(3M+H)^+$, 875 $(2M+H)^+$, 438 $(M+H)^+$, 309 $(S)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 1310 $(2M-H)^-$, 873 $(2M-H)^-$, 436 $(M-H)^-$, 128 $(B)^-$, 95 $(CH_3SO_3)^-$; $[\alpha]_D^{20}$ = -84 (*c*, 0.84 DMSO).

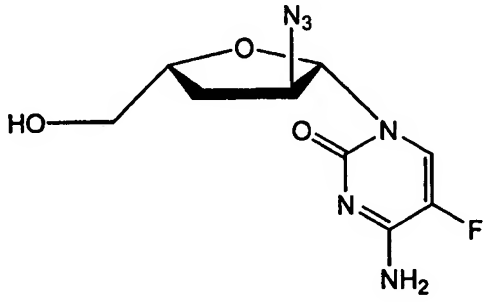
1-(2,3-Dideoxy-2-azido-5-O-*t*-butyldimethylsilyl-β-L-*erythro*pentofuranosyl)-5-fluorocytosine 21



To a solution of **20** (442 mg, 1.01 mmol) in anhydrous dilmethylformamide (12 mL) was added lithium azide moistened with 10% methanol (265 mg, 4.87 mmol). The reaction mixture was stirred at 100°C during 2.5 h, and then cooled to room temperature, poured onto ice/water (200 mL) and extracted with chloroform (3× 100 mL). Combined extracts were washed with a saturated aqueous sodium hydrogen carbonate solution (2× 100 mL), with water (5× 100 mL) and then dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : methanol (4%) in chloroform] to give pure **21** (291 mg, 75% yield) as a white solid: mp 147-148°C ;

UV (ethanol): λ_{max} 242 nm (ε 7700), 283 nm (ε 7400), λ_{min} 226 nm (ε 6600), 264 nm (ε 5800); ^{1}H NMR (DMSO-d_6): δ 8.05 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.0 Hz), 7.9-7.4 (br d, 2H, NH_2), 5.7 (br s, 1H, H-1'), 4.37 (d, 1H, H-2', $J_{2'\text{-}3'}$ = 5.5 Hz), 4.3 (m, 1H, H-4'), 4. (m, 1H, H-5'), 3.7 (m, 1H,H-5"), 2.0 (m, 1H, H-3'), 1.8 (m, 1H, H-3"), 0.88 (s, 9H, $(CH_3)_3C$-Si), 0.05 (s, 6H, $(CH_3)_2Si$); MS (matrix GT): FAB>0 *m/z* 769 $(2M+H)^+$, 385 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 1151 $(3M-H)^-$, 767 $(2M-H)^-$, 383 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = +25 (*c*, 0.95 DMSO).

1-(2,3-Dideoxy-2-azido-β-L-*erythro*-pentofuranosyl)-5-fluorocytosine 22 (2'-N_3-β-L-5-FddC)



A 1 M solution of tetrabutylammonium trifluoride in tetrahydrofurane (TBAF/THF, 1.90 mL, 1.90 mmol) was added to a solution of 21 (480 mg, 1.25 mmol) in anhydrous THF (8 mL). The resulting mixture was stirred at room temperature for 1.5 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [eluent : stepwise gradient of methanol (4-8%) in chloroform]. Finally, the appropriate fractions were evaporated under reduced pressure, diluted with methanol and filtered through a unit Millex HV-4 (0.45 μm, Millipore) to give pure 22 (304 mg, 90% yield), which was crystallized from ethanol: mp 219-221°C; UV (ethanol): λ_{max} 241 nm (ε 7700), 284 nm (ε 7300), λ_{min} 225 nm (ε 6500), 263 nm (ε 5400); ^{1}H NMR (DMSO-d_6): δ 8.31 (d, 1H, H-6, $J_{6\text{-}F5}$ = 7.4 Hz), 7.9-7.4 (br d, 2H, NH_2), 5.65 (m, 1H, H-1'), 5.32 (br s, 1H, OH-5'), 4.35 (d, 1H, H-2', $J_{2'\text{-}3'}$ = 5.6 Hz), 4.2 (m, 1H, H-4'), 3.8 (m, 1H, H-5'), 3.6 (m, 1H, H-5"), 2.1 (m, 1H, H-3'), 1.8 (m, 1H, H-2"); MS (matrix GT): FAB>0 *m/z* 541 $(2M+H)^+$, 363 $(M+G+H)^+$, 271 $(M+H)^+$, 130 $(BH_2)^+$; FAB<0 *m/z* 539 $(2M-H)^-$, 269 $(M-H)^-$, 128 $(B)^-$; $[\alpha]_D^{20}$ = +29 (*c*, 0.85 DMSO); Anal. ($C_9H_{11}FN_6O_3$) C, H, N, F.

Analytical data

Compd	Formula	Anal. calculated				Anal. found			
		C	H	N	F	C	H	N	F
13	$C_{18}H_{17}FN_2O_7$, $^2/_3$ C_7H_8	59.99	4.96	6.18	4.19	59.60	4.96	6.02	3.76
14	$C_{16}H_{15}FN_2O_6$	54.86	4.32	8.00	5.42	54.75	4.16	7.78	5.49
22	$C_9H_{11}FN_6O_3$	40.00	4.10	31.10	7.03	40.07	4.16	31.10	6.99

Anti-HIV Activity of the Active Compounds

Antiviral compositions can be screened in vitro for inhibition of HIV by various experimental techniques. One such technique involves measuring the inhibition of viral replication in human peripheral blood mononuclear (PBM) cells. The amount of virus produced is determined by measuring the quantity of virus-coded reverse transcriptase (RT), an enzyme found in retroviruses, that is present in the cell culture medium.

Three-day-old phytohemagglutinin-stimulated PBM cells (10^6 cells/ml) from hepatitis B and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 times the 50% tissue culture infectious done (TICD 50) per ml and cultured in the presence and absence of various concentrations of antiviral compounds.

Approximately one hour after infection, the medium, with the compound to be tested (2 times the final concentration in medium) or without compound, was added to the flasks (5 ml; final volume 10 ml). AZT was used as a positive control. The cells were exposed to the virus (about 2 x 10^5 dpm/ml, as determined by reverse transcriptase assay) and then placed in a CO_2 incubator. HIV-1 (strain LAV) was obtained from the Centers for Disease Control, Atlanta, Georgia. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal et al. (J. Immun. Meth. 76, 171-183, 1985) and Spira et al., (J. Clin. Meth. 25, 97-99, 1987), except that fungizone was not included in the medium (see Schinazi, et al., Antimicrob. Agents Chemother. 32, 1784-1787 (1988); Antimicrob. Agents Chemother., 34: 1061-1067 (1990)).

On day 6, the cells and supernatant were transferred to a 15 ml tube and centrifuged at about 900 g for 10 minutes. Five ml of supernatant were removed and the virus was concentrated by centrifugation at 40,000 rpm for 30 minutes (Beckman 70.1 Ti rotor). The solubilized virus pellet was processed for determination of the levels of reverse transcriptase. Results are expressed in dpm/ml of sampled supernatant. Virus from smaller volumes of supernatant (1 ml) can also be concentrated by centrifugation prior to solubilization and determination of reverse transcriptase levels.

The median effective (EC_{50}) concentration was determined by the median effect method (Antimicrob. Agents Chemother. 30, 491-498 (1986). Briefly, the percent inhibition of virus, as determined from measurements of reverse transcriptase, is plotted versus the

micromolar concentration of compound. The EC_{50} is the concentration of compound at which ther is a 50% inhibition of viral growth.

Mitogen stimulated uninfected human PBM cells (3.8×10^5 cells/ml) were cultured in the presence and absence of drug under similar conditions as those used for the antiviral assay described above. The cells were counted after six days using a hemacytometer and the trypan blue exclusion method, as described by Schinazi et al., Antimicrobial Agents and Chemotherapy, 22(3), 499 (1982). The IC_{50} is the concentration of compound which inhibits 50% of normal cell growth.

Example 3 Anti-HIV Activity of β-L-(2' or 3')-A-5-FddC

The anti-HIV activity of L-2'-A-5-FddC and L-3'-A-5-FddC was tested in CEM and PBM cells. The results are provided in Table 1.

Table 1

Compound	Antiviral Activity EC_{50} (μM)	Cytotoxicity $I\ C_{50}$ (μM)	Selectivity Index IC_{50}/EC_{50}
L-2'-A-5-FddC (CEM)	3.90	>100	>30
L-3'-A-5-FddC (CEM)	0.29	>100	>344
L-2'-A-5-FddC (PBM)	1.00	>100	>100
L-3'-A-5-FddC (PBM)	0.05	>100	>2647

Preparation of Pharmaceutical Compositions

Humans suffering from any of the disorders described herein, including AIDS, can be treated by administering to the patient an effective treatment amount of β-L-(2' or 3')-A-5-FddC as described herein, or a pharmaceutically acceptable prodrug or salt thereof in the

presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of compound to inhibit viral replication *in vivo,* without causing serious toxic effects in the patient treated.

By "inhibitory amount" is meant an amount of active ingredient sufficient to exert an inhibitory effect as measured by, for example, an assay such as the ones described herein.

A preferred dose of the compound for all of the above mentioned conditions will be in the range from about 1 to 50 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable prodrug can be calculated based on the weight of the parent nucleoside to be delivered. If the prodrug exhibits activity in itself, the effective dosage can be estimated as above using the weight of the prodrug, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually convenient, and more typically 50 to 500 mg.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μM, preferably about 1.0 to 10 μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient

may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The compound can be administered as a component of an elixir, suspension, syrup, water, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The compound or a pharmaceutically acceptable derivative or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, protease inhibitors, or other nucleoside or nonnucleoside antiviral agents. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium

chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

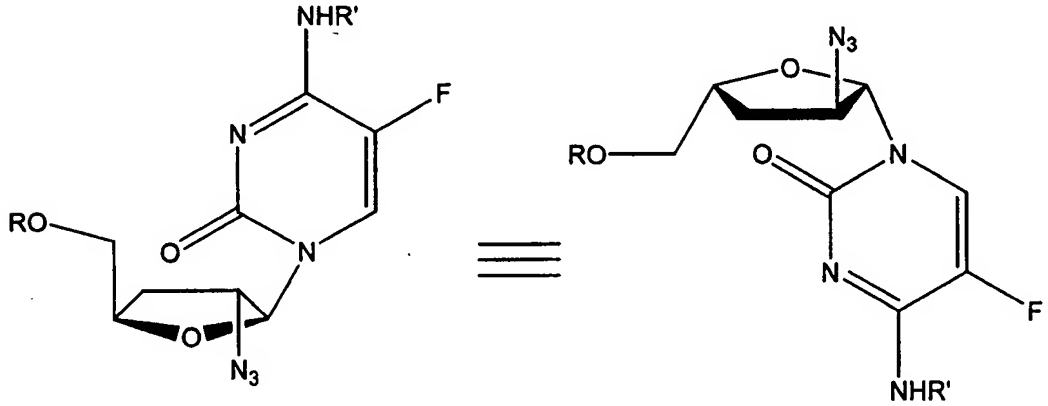
In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylacetic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of this invention.

We claim:

1. A method for the treatment of HIV infection in a host comprising administering an effective amount of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl.

2. The method of claim 1, wherein R is H.

3. The method of claim 1, wherein R is acyl.

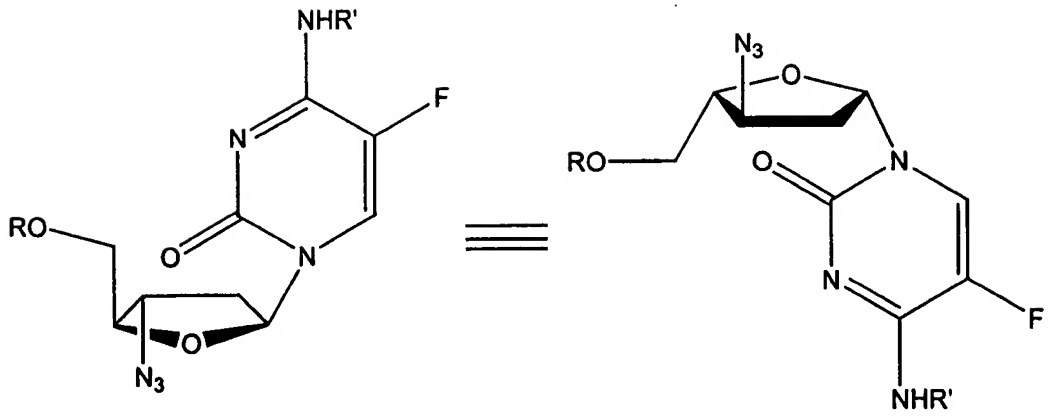
4. The method of claim 1, wherein R is monophosphate.

5. The method of claim 1, wherein R is diphosphate.

6. The method of claim 1, wherein R is triphosphate.

7. The method of claim 1, wherein R is a stabilized phosphate derivative.

8. A method for the treatment of HIV infection in a host comprising administering an effective amount of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), R' is H, acyl, or alkyl.

9. The method of claim 8, wherein R is H.

10. The method of claim 8, wherein R is acyl.

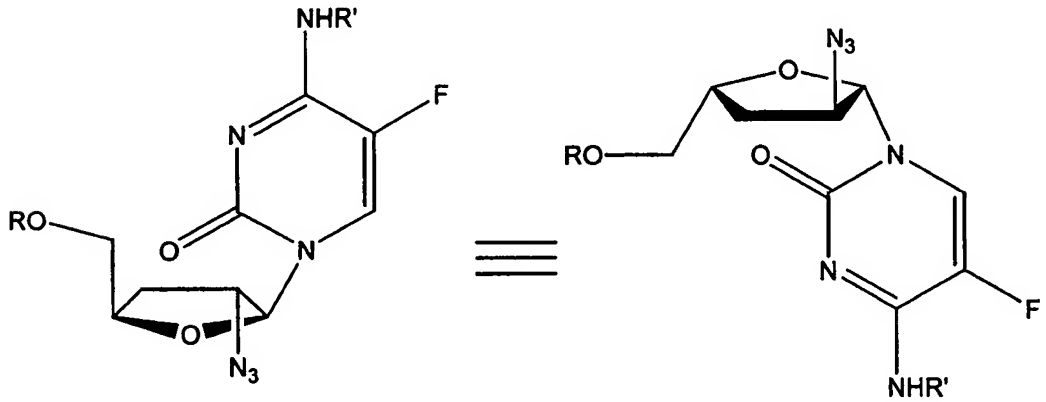
11. The method of claim 8, wherein R is monophosphate.

12. The method of claim 8, wherein R is diphosphate.

13. The method of claim 8, wherein R is triphosphate.

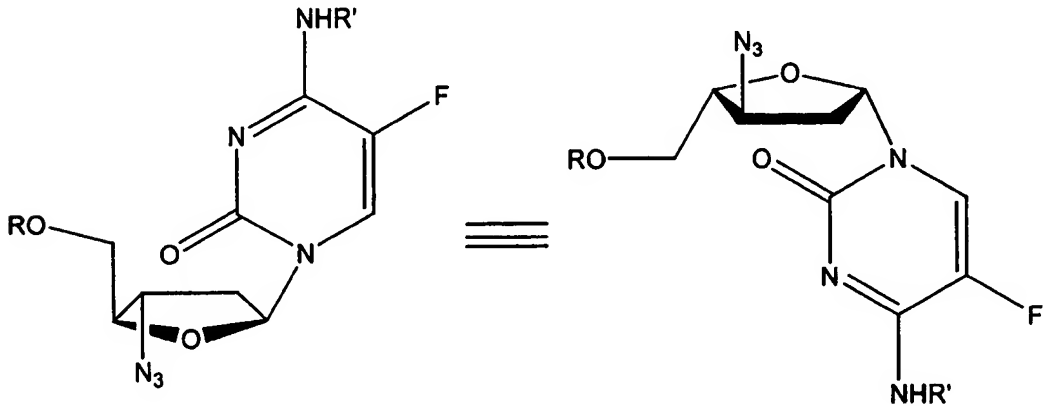
14. The method of claim 8, wherein R is a stabilized phosphate derivative.

15. Use of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



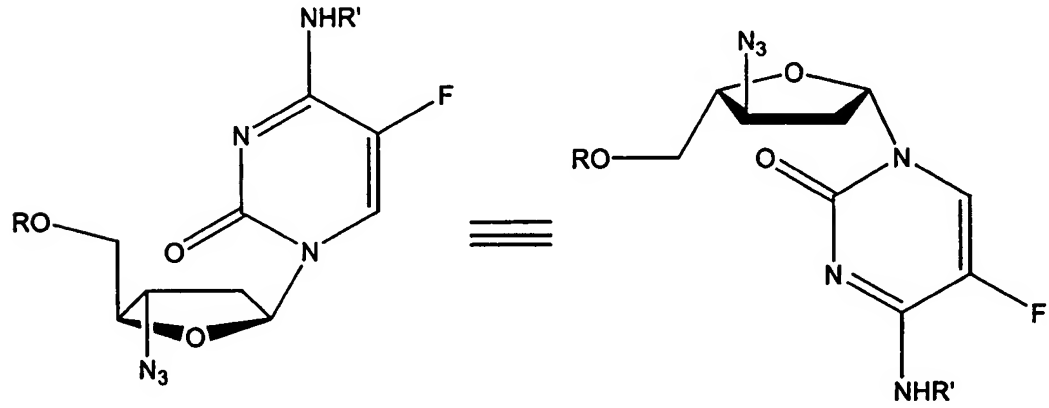
wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl, for the treatment of HIV infection in a human or other host animal.

16. Use of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



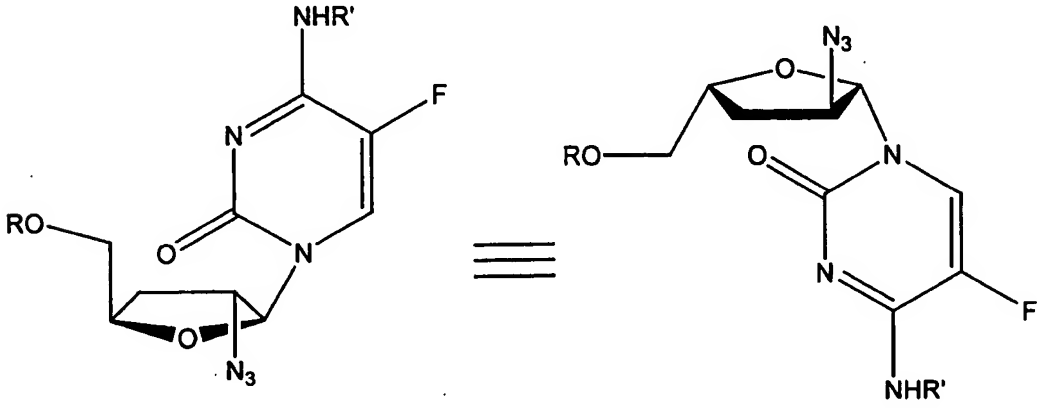
wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl for the treatment of HIV in a human or other host animal.

17. Use of a β-L-(3'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl for the manufacture of a medicament for the treatment of HIV in a human or other host animal.

18. Use of a β-L-(2'-azido)-2',3'-dideoxy-5-fluorocytosine compound or a pharmaceutically acceptable ester, salt or prodrug thereof of the formula:



wherein R is H, acyl, monophosphate, diphosphate, or triphosphate, or a stabilized phosphate derivative (to form a stabilized nucleotide prodrug), and R' is H, acyl, or alkyl, for the manufacture of a medicament for the treatment of HIV infection in a human or other host animal.

19. The method of claim 1, wherein R' is H, and R is H.

20. The method of claim 1, wherein R' is H.

21. The method of claim 1, wherein R' is acyl, and R is H.

22. The method of claim 1, wherein R' is acyl.

23. The method of claim 1, wherein R' is alkyl, and R is H.

24. The method of claim 1, wherein R' is alkyl.

25. The method of claim 8, wherein R' is H, and R is H.

26. The method of claim 8, wherein R' is H.

27. The method of claim 8, wherein R' is acyl, and R is H.

28. The method of claim 8, wherein R' is acyl.

29. The method of claim 8, wherein R' is alkyl, and R is H.

30. The method of claim 8, wherein R' is alkyl.

General scheme for the sterospecific synthesis of 3'-substituted β-L-dideoxynucleosides

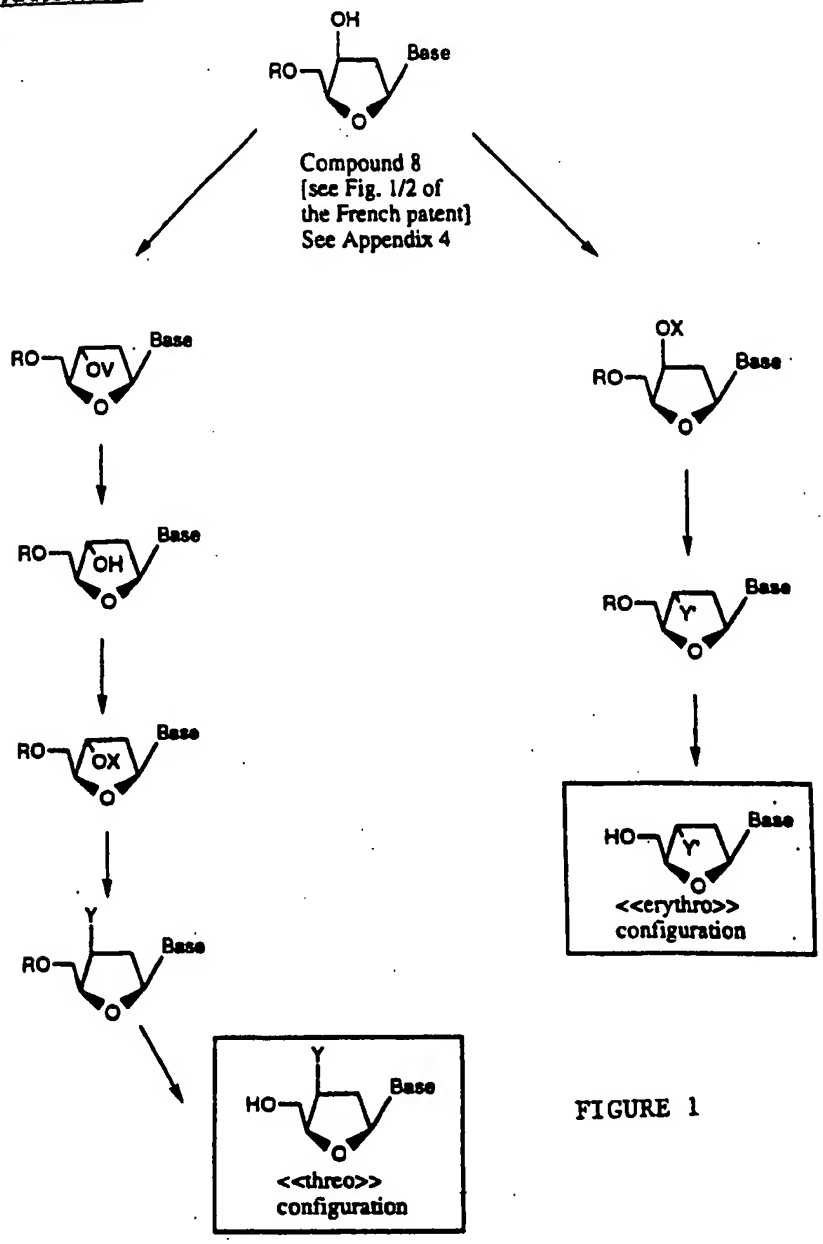


FIGURE 1

V = acyl (CH_3-C, C_6H_5-C)

X = Leaving group [$CH_3\ SO_2$, $CH_3\ C_6H_4\ SO_2$, $CF_3\ SO_2$]

Y, Y' = F, N_3, NR_1R_2 [R_1,R_2 = H, alkyl, aryl],

NO_2, NOR [R = H, alkyl, acyl], O-alkyl, O-aryl, etc.

General Scheme for the Stereospecific Synthesis of 2'-substituted β-L-dideoxynucleosides

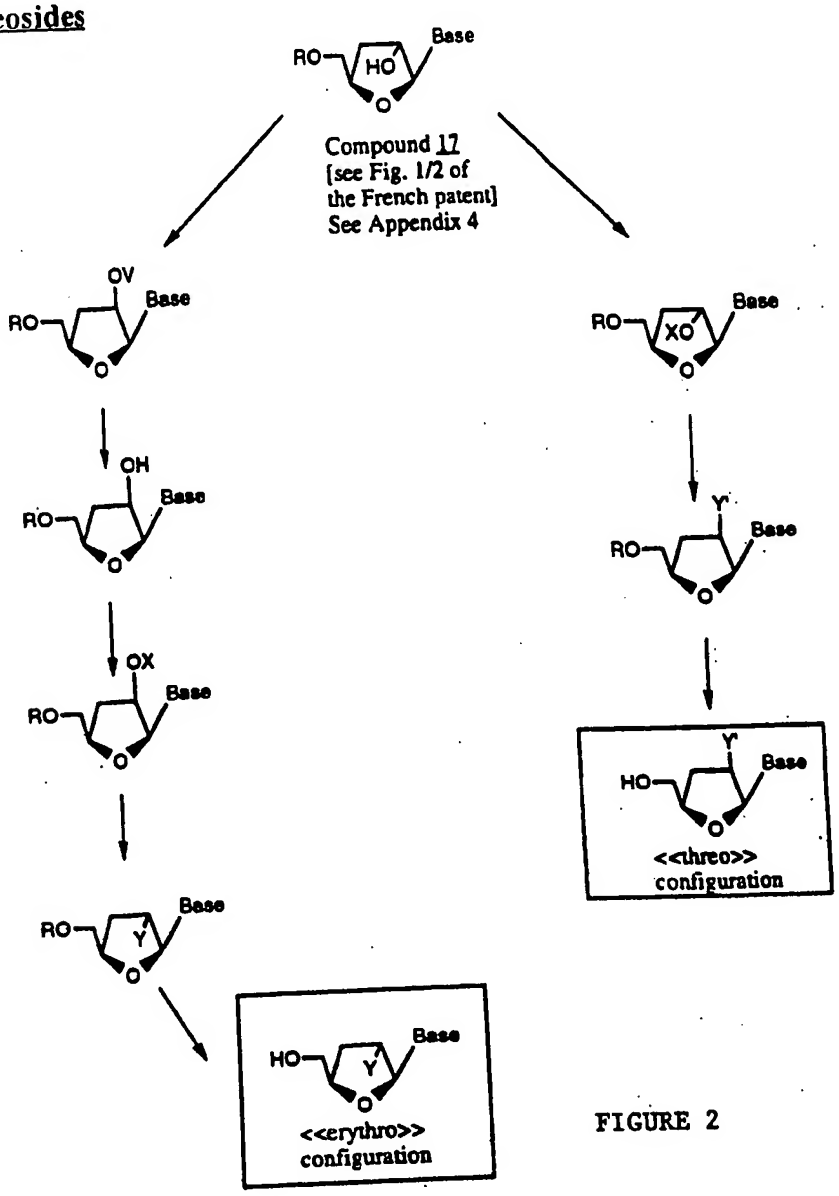


FIGURE 2

V = acyl [CH_3-C C_6H_5-C]

X = Leaving group [CH_3 SO_2, CH_3 $C_6H_4SO_2$,H, CF_3 SO_2]

Y, Y' = F, N_3, NR_1R_2 [R_1,R_2 = H, alkyl, aryl],

NO_2, NOR [R = H, alkyl, acyl], O-alkyl, O-aryl, etc.

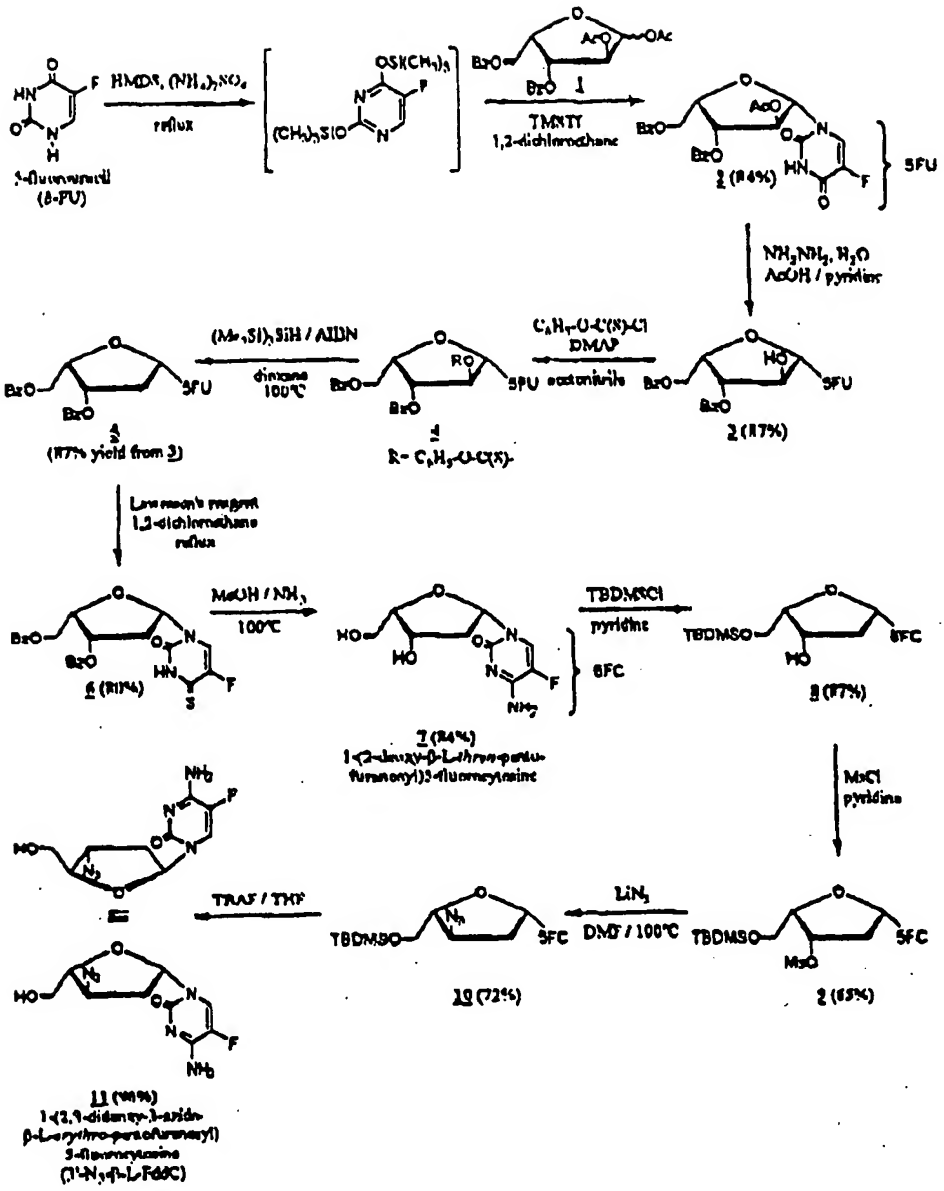
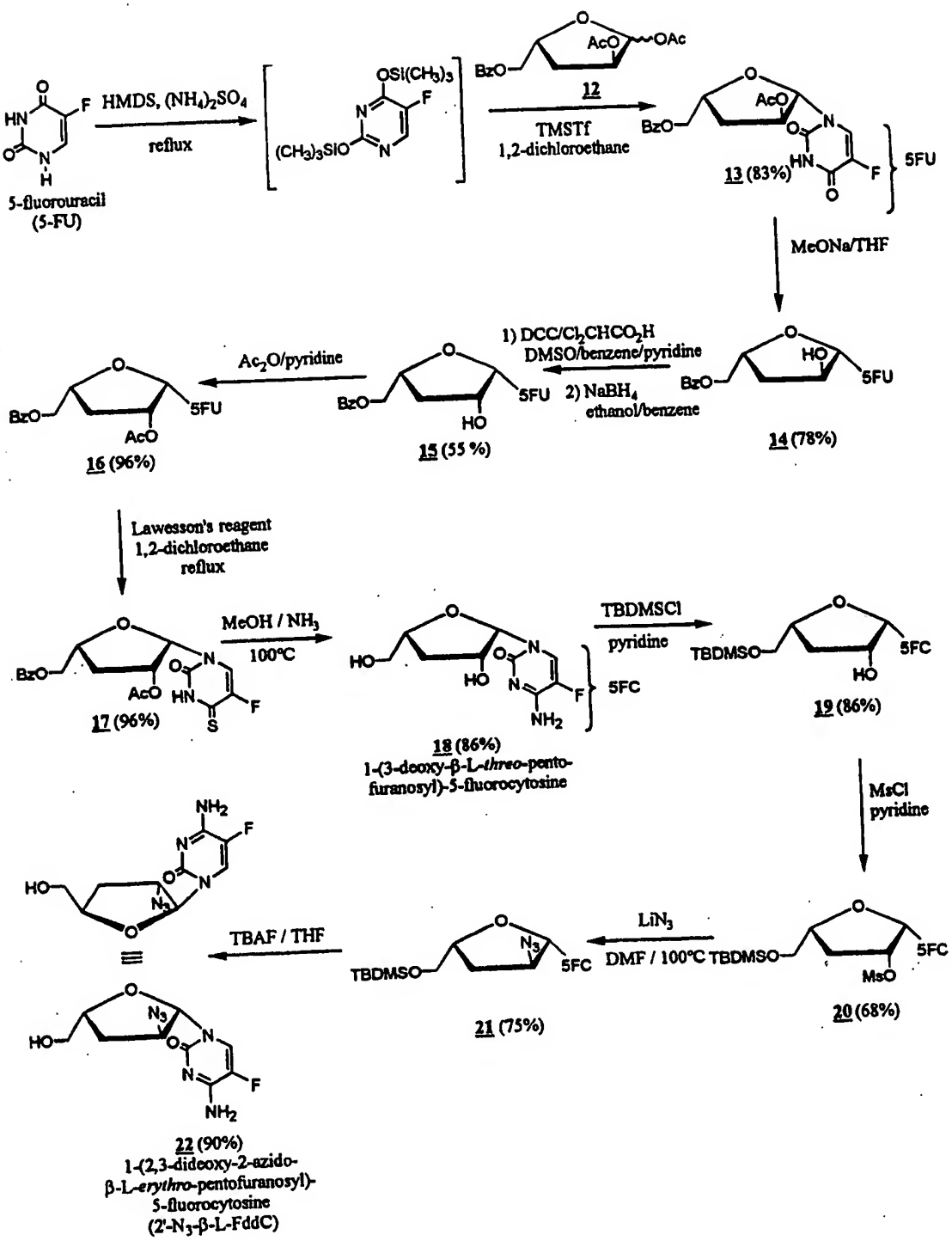


Figure 3

Figure 4


5-fluorouracil
(5-FU)
HMDS, $(NH_4)_2SO_4$
reflux
TMSTf
1,2-dichloroethane
12
13 (83%)
MeONa/THF
1) DCC/Cl_2CHCO_2H
DMSO/benzene/pyridine
2) $NaBH_4$
ethanol/benzene
14 (78%)
15 (55 %)
Ac_2O/pyridine
16 (96%)
Lawesson's reagent
1,2-dichloroethane
reflux
17 (96%)
MeOH / NH_3
100°C
18 (86%)
1-(3-deoxy-β-L-*threo*-pento-furanosyl)-5-fluorocytosine
TBDMSCl
pyridine
19 (86%)
MsCl
pyridine
20 (68%)
LiN_3
DMF / 100°C
21 (75%)
TBAF / THF
22 (90%)
1-(2,3-dideoxy-2-azido-β-L-*erythro*-pentofuranosyl)-5-fluorocytosine
(2'-N_3-β-L-FddC)

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/US 99/26156

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/7068 A61P31/18 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 07086 A (UNIV EMORY ;CENTRE NAT RECH SCIENT (FR); UAB RESEARCH FOUNDATION () 16 March 1995 (1995-03-16) figure 1 page 6, line 3 - line 10 page 9, line 3 - line 4 page 9, line 10 -page 10, line 9 claims	1-30
Y	WO 97 09052 A (MANOUILOV KOSTANTIN ;CHU CHUNG K (US); JINFA DU (US); KOTRA LAKSHI) 13 March 1997 (1997-03-13) abstract page 9, line 22 -page 10, line 6 page 16, line 26 -page 17, line 7	1-30

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2000	16/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Villa Riva, A

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INTERNATIONAL SEARCH REPORT

Int... ...nal Application No
PCT/US 99/26156

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 16186 A (ICN PHARMACEUTICALS ;AVERETT DEVRON (US); TAM ROBERT (US); RAMASAM) 23 April 1998 (1998-04-23) page 3, line 25 - line 26 formula I	1-30
Y	LIN TS ET AL : "Synthesis and Antiviral Activity of Various 3'-Azido Analogues of Pyrimidine Deoxyribonucleosides against Human Immunodeficiency Virus" J MED CHEM, vol. 31, 1988, pages 336-340, XP002131011 Chart I, compound 19 table I	1-30
Y	GOSSELIN G ET AL: "Anti HIV Activities of the beta-L Enantiomer of 2',3'-Dideoxycytidine and Its 5-Fluoro Derivative In Vitro " ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 38, no. 6, June 1994 (1994-06), pages 1292-1297, XP000872743 tables 2,3 page 1295, right-hand column, line 29 - line 38	1-30

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. nal Application No
PCT/US 99/26156

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9507086 A	16-03-1995	AU 7954694 A	27-03-1995
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		JP 9504785 T	13-05-1997
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(71) Applicants *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **ISIS PHARMACEUTICALS, INC.** [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **OLSEN, David, B.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **LAFEMINA, Robert, L.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **ELDRUP, Anne, B.** [DK/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US). **BERA, Sanjib** [IN/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).

(74) Common Representative: **DURETTE, Philippe, L.** et al.; Merck & Co., Inc., 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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WO 03/068244 A1

(54) Title: METHODS OF INHIBITING ORTHOPOXVIRUS REPLICATION WITH NUCLEOSIDE COMPOUNDS

(57) Abstract: The present invention provides methods of inhibiting orthopoxvirus replication and/or treating orthopoxvirus infection with certain nucleoside compounds and derivatives thereof. These compounds are particularly useful as inhibitors of vaccinia virus and variola virus replication and/or for the treatment of vaccinia virus and variola virus infection. The nucleoside compounds may be administered alone or in combination with other agents active against orthopoxvirus infection, in particular against vaccinia virus or variola virus infection. Another aspect of the present invention provides for the use of such nucleoside compounds in the manufacture of a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection. Yet a further aspect of the present invention provides such nucleoside compounds for use as a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection.

TITLE OF THE INVENTION

METHODS OF INHIBITING ORTHOPOXVIRUS REPLICATION WITH NUCLEOSIDE COMPOUNDS

FIELD OF THE INVENTION

The present invention is concerned with methods of inhibiting orthopoxvirus replication and methods for treating orthopoxvirus infections with certain nucleoside compounds and derivatives thereof. The compounds are particularly useful for inhibiting the replication of vaccinia, variola, cowpox, and monkeypox virus and for the treatment of vaccinia, variola, cowpox, and monkeypox virus infections. Another aspect of the present invention provides for the use of the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection. Yet a further aspect of the present invention provides for the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of orthopoxvirus replication and/or for the treatment of orthopoxvirus infection.

BACKGROUND OF THE INVENTION

Orthopoxvirus is a genus of the *Poxviridae* family of complex DNA viruses that replicate in the cytoplasm of vertebrate and invertebrate cells. The Poxviridae family is characterized by having a large complex virion containing enzymes that synthesize mRNA, a genome composed of a single linear double-stranded DNA molecule of 130-300 kilobase pairs with a hairpin loop at each end, and a cytoplasmic site of replication. Members of the orthopoxvirus genus include cowpox, monkeypox, vaccinia, and variola virus [for a description of the *Poxviridae* family, reference is made to B. Moss, "Poxviridae: The Viruses and Their Replication," in Fields Virology, B.N. Fields, et al., Eds., 3rd ed., Ch. 83, pages 2637-2671 (1996)]. Variola virus is the agent responsible for smallpox infections. Smallpox infections were effectively eradicated subsequent to the introduction of prophylactic vaccinations with cowpox and vaccinia virus. However, most of the human populations worldwide are no longer immune to smallpox as a result of the discontinuation of routine vacination in the early 1980's.

There are very few compounds available as therapeutics against orthopoxvirus infections. Two drugs under investigation are cidofovir and ribavirin.

Cidofovir is the generic name for (S)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine [(S)-HPMPC] which is currently the leading agent for the treatment of orthopoxvirus infections in humans. It is potent against vaccinia and cowpox virus infection in mice when administered subcutaneously or intraperitoneally. However, cidofovir's therapeutic utility is limited by safety concerns as well as lack of oral bioavailablity [see D.F. Smee et al., "Effects of cidofovir on the pathogenesis of a lethal vaccinia virus respiratory infection in mice," Antiviral Res., 52: 55-62 (2001) and references cited therein]. Inhibition of vaccinia virus is considered in the art to be predictive of inhibitory activity against other orthopoxviruses, including variola; see E. De Clercq, "Vaccinia Virus Inhibitors as a Paradigm for the Chemotherapy of Poxvirus Infections," Clin. Microbiol. Rev., 14: 382-397 (2001).

Ribavirin has also been found to inhibit vaccinia virus and other orthopoxvirus replication in cell culture (see J.H. Huffman et al., "In vitro effect of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide on DNA and RNA viruses," Antimicrobial Agents and Chemotherapy, 3: 235-241 (1973) and D.F. Smee et al., "Potential of the IMP dehydrogenase inhibitors for antiviral therapies of poxvirus infections," Antiviral Res., 37: A89 (1998)]. Ribavirin was also reported to suppress vaccinia virus-induced lesions in a mouse model and to effectively treat vaccinia keratitis in rabbits. However, ribavirin causes anemia during prolonged administration and at high doses has certain immunosuppressive properties limiting its clinical usefulness against orthopoxvirus.

Consequently, there exists a need for more effective anti-orthopoxvirus agents particularly as a result of the threat of either variola (smallpox) or monkeypox viruses in biowarfare or bioterrorism. Preferably such agents should be effective when administered orally and be safe and well-tolerated by the host.

It has now been found that nucleoside compounds of the present invention and certain derivatives thereof are potent inhibitors of orthopoxvirus replication and in particular of vaccinia, variola, cowpox, and monkeypox virus replication. The instant nucleoside compounds and derivatives thereof are useful to treat orthopoxvirus infection and in particular vaccinia, variola, cowpox, and monkeypox virus infection.

It is therefore an object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful as inhibitors of the replication of orthopoxvirus and in particular as inhibitors of the replication of vaccinia, variola, cowpox, and monkeypox virus.

It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof which are useful in the treatment of orthopoxvirus infection and in particular in the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds of the present invention in association with a pharmaceutically acceptable carrier.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives of the present invention for use as inhibitors of orthopoxvirus replication and in particular as inhibitors of vaccinia, variola, cowpox, and monkeypox virus replication.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives of the present invention for use in the treatment of orthopoxvirus infection and in particular in the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide pharmaceutical compositions comprising the nucleoside compounds and derivatives of the present invention in combination with other agents active against orthopoxvirus and in particular against vaccinia, variola, cowpox, and monkeypox virus.

It is another object of the present invention to provide methods for the inhibition of orthopoxvirus replication and in particular for the inhibition of vaccinia, variola, cowpox, and monkeypox virus replication.

It is another object of the present invention to provide methods for the treatment of orthopoxvirus infection and in particular for the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide methods for the treatment of orthopoxvirus infection in combination with other agents active against orthopoxvirus and in particular for the treatment of vaccinia, variola, cowpox, and monkeypox virus infection in combination with other agents active against vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide nucleoside compounds and certain derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of orthopoxvirus replication and/or the treatment of orthopoxvirus infection and in particular for the inhibition of vaccinia,

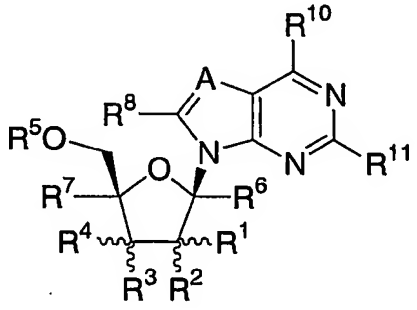
variola, cowpox, and monkeypox virus replication and/or the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

It is another object of the present invention to provide for the use of the nucleoside compounds and certain derivatives thereof of the present invention and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of orthopoxvirus replication and/or the treatment of orthopoxvirus infection and in particular for the inhibition of vaccinia, variola, cowpox, and monkeypox virus replication and/or the treatment of vaccinia, variola, cowpox, and monkeypox virus infection.

These and other objects will become readily apparent from the detailed description which follows.

SUMMARY OF THE INVENTION

The present invention provides a method for inhibiting orthopoxvirus replication and/or a method for treating orthopoxvirus infection in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is amino, fluorine, hydroxy, C_{1-10} alkylcarbonyloxy, mercapto, or C_{1-4} alkoxy;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, C_{2-18} alkenylcarbonyloxy, C_{1-10} alkyloxycarbonyloxy, C_{3-6} cycloalkylcarbonyloxy, C_{3-6} cycloalkyloxycarbonyloxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, C_{2-18} alkenylcarbonyl, C_{1-10} alkyloxycarbonyl, C_{3-6} cycloalkylcarbonyl, C_{3-6} cycloalkyloxycarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

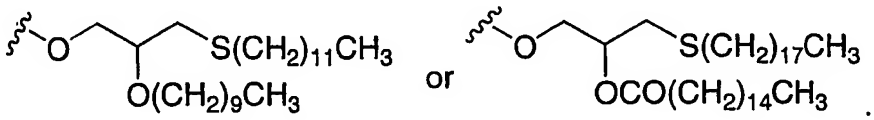
R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, or (C_{1-4} alkyl)$_{0-2}$ aminomethyl;

R^9 is hydrogen, cyano, nitro, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, or C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

R^{10} and R^{11} are each independently hydrogen, hydroxy, mercapto, halogen, C_{1-4} alkoxy, C_{1-4} alkylthio, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, phenyl-C_{1-2} alkylamino, C_{1-4} acylamino, C_{1-8} alkylcarbonyloxy, or $OCH(C_{1-4}$ alkyl)$O(C=O)C_{1-4}$ alkyl;

each R^{12} is independently hydrogen or C_{1-6} alkyl; and

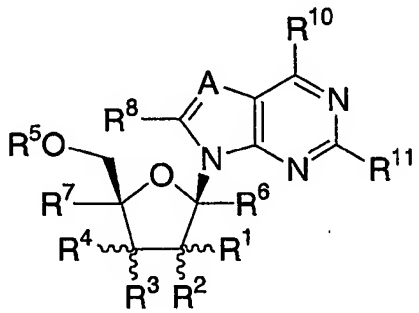
R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl)$O(C=O)C_{1-4}$ alkyl,



Also encompassed within the present invention are pharmaceutical compositions containing the compounds alone or in combination with other agents active against orthopoxvirus and in particular against vaccinia, variola, cowpox, and monkeypox virus as well as methods for the inhibition of orthopoxvirus replication and for the treatment of orthopoxvirus infection with such compositions.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for inhibiting orthopoxvirus replication and/or a method for treating orthopoxvirus infection in a mammal in need thereof comprising administering to the mammal a therapeutically effective amount of a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is amino, fluorine, hydroxy, C_{1-10} alkylcarbonyloxy, mercapto, or C_{1-4} alkoxy;

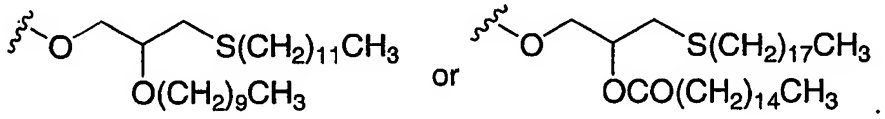
R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, C_{2-18} alkenylcarbonyloxy, C_{1-10} alkyloxycarbonyloxy, C_{3-6} cycloalkylcarbonyloxy, C_{3-6} cycloalkyloxycarbonyloxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, C_{2-18} alkenylcarbonyl, C_{1-10} alkyloxycarbonyl, C_{3-6} cycloalkylcarbonyl, C_{3-6} cycloalkyloxycarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

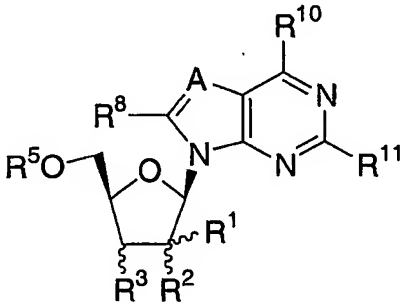
R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy,

C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, or (C_{1-4} alkyl)$_{0-2}$ aminomethyl;
R^9 is hydrogen, cyano, nitro, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, or C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;
R^{10} and R^{11} are each independently hydrogen, hydroxy, mercapto, halogen, C_{1-4} alkoxy, C_{1-4} alkylthio, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, phenyl-C_{1-2} alkylamino, C_{1-4} acylamino, C_{1-8} alkylcarbonyloxy, or $OCH(C_{1-4}$ alkyl)$O(C=O)C_{1-4}$ alkyl;
each R^{12} is independently hydrogen or C_{1-6} alkyl; and
R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl)$O(C=O)C_{1-4}$ alkyl,



In one embodiment of the present invention is the method of inhibiting orthopoxvirus replication and/or treating orthopoxvirus infection with a compound of structural formula II which is of the stereochemical configuration:



(II)

or a pharmaceutically acceptable salt thereof; wherein
A is N or C-R^9;
R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;

R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or C_{1-3} alkoxy;
R^3 is hydrogen, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or C_{1-3} alkoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;
R^8 is hydrogen, amino, or C_{1-4} alkylamino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

In a second embodiment of the present invention is the method of inhibiting orthopoxvirus replication and/or treating orthopoxvirus infection with a compound of structural formula II wherein
R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;
R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or methoxy;
R^3 is hydrogen, fluoro, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or methoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, or $P_3O_9H_4$;
R^8 is hydrogen or amino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

Illustrative of the invention is a method for inhibiting orthopoxvirus replication and/or treating orthopox infection wherein the compound is selected from:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-hydroxypurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-cyclopropylaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2,6-diaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-methylaminopurine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

Further illustrative of the invention is a method for inhibiting orthopoxvirus replication and/or treating orthopox infection wherein the compound is selected from:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and

4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

In a third embodiment of the methods of the present invention, the orthopoxvirus replication is selected from the group consisting of vaccinia virus replication, variola virus replication, cowpox virus replication, and monkeypox virus replication. In a class of this embodiment, the orthopoxvirus replication is vaccinia virus replication or variola virus replication.

In a fourth embodiment of the methods of the present invention, the orthopoxvirus infection is selected from the group consisting of vaccinia virus infection, variola virus infection, cowpox virus infection, and monkeypox virus infection. In a class of this embodiment, the orthopoxvirus infection is vaccinia virus infection or variola virus infection.

Another aspect of the present invention provides the novel nucleoside derivative, 6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine or a pharmaceutically acceptable salt thereof.

Throughout the instant application, the following terms have the indicated meanings:

The alkyl groups specified above are intended to include those alkyl groups of the designated length in either a straight or branched configuration. Exemplary of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, and the like.

The term "alkenyl" shall mean straight or branched chain alkenes of two to six total carbon atoms, or any number within this range (e.g., ethenyl, propenyl, butenyl, pentenyl, etc.).

The term "alkynyl" shall mean straight or branched chain alkynes of two to six total carbon atoms, or any number within this range (e.g., ethynyl, propynyl, butynyl, pentynyl, etc.).

The term "cycloalkyl" shall mean cyclic rings of alkanes of three to eight total carbon atoms, or any number within this range (i.e., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl).

The term "alkoxy" refers to straight or branched chain alkoxides of the number of carbon atoms specified (e.g., C_{1-4} alkoxy), or any number within this range [i.e., methoxy (MeO-), ethoxy, isopropoxy, etc.].

The term "alkylthio" refers to straight or branched chain alkylsulfides of the number of carbon atoms specified (e.g., C_{1-4} alkylthio), or any number within this range [i.e., methylthio (MeS-), ethylthio, isopropylthio, etc.].

The term "alkylamino" refers to straight or branched alkylamines of the number of carbon atoms specified (e.g., C_{1-4} alkylamino), or any number within this range [i.e., methylamino, ethylamino, isopropylamino, t-butylamino, etc.].

The term "alkylsulfonyl" refers to straight or branched chain alkylsulfones of the number of carbon atoms specified (e.g., C_{1-6} alkylsulfonyl), or any number within this range [i.e., methylsulfonyl ($MeSO_2$-), ethylsulfonyl, isopropylsulfonyl, etc.].

The term "alkyloxycarbonyl" refers to straight or branched chain esters of a carboxylic acid derivative of the present invention of the number of carbon atoms specified (e.g., C_{1-4} alkyloxycarbonyl), or any number within this range [i.e., methyloxycarbonyl (MeOCO-), ethyloxycarbonyl, or butyloxycarbonyl].

The term "alkyloxycarbonyloxy" refers to straight or branched chain alkyl carbonates of the present invention of the number of carbon atoms specified (e.g., C_{1-10} alkyloxycarbonyloxy), or any number within this range [i.e., methyloxycarbonyloxy (MeOCOO-), ethyloxycarbonyloxy, or butyloxycarbonyloxy].

The term "alkylcarbonyloxy" refers to straight or branched chain alkanoic acid derivatives of alcohols of the present invention of the number of carbon atoms specified (e.g., C_{1-16} alkylcarbonyloxy), or any number within this range [i.e., methylcarbonyloxy (MeCOO-), ethylcarbonyloxy, or n-octylcarbonyloxy].

The term "cycloalkylcarbonyloxy" refers to cyclic alkanoic acid derivatives of alcohols of the present invention of the number of carbon atoms specified (e.g., C_{3-6} cycloalkylcarbonyloxy), or any number within this range [i.e., cyclopropylcarbonyloxy, cyclopentylcarbonyloxy, or cyclohexylcarbonyloxy].

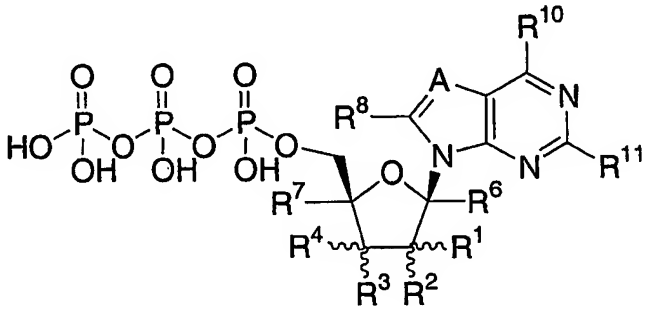
The term "alkenylcarbonyloxy" refers to a straight or branched chain alkenoic acid derivatives of alcohols of the present invention having two to eighteen total carbon atoms and containing one to three double bonds in the alkene chain.

The term "aryl" includes both phenyl, naphthyl, and pyridyl. The aryl group is optionally substituted with one to three groups independently selected from C_{1-4} alkyl, halogen, cyano, nitro, trifluoromethyl, C_{1-4} alkoxy, and C_{1-4} alkylthio.

The term "halogen" is intended to include the halogen atoms fluorine, chlorine, bromine and iodine.

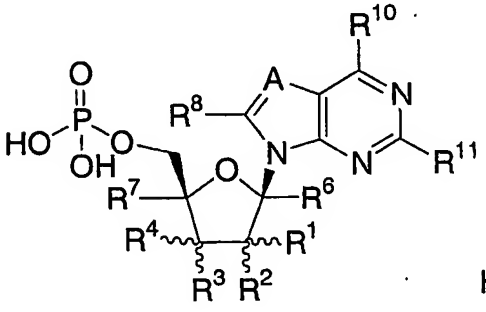
The term "substituted" shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or plurally.

The term "5'-triphosphate" refers to a triphosphoric acid ester derivative of the 5'-hydroxyl group of a nucleoside compound of the present invention having the following general structural formula III:

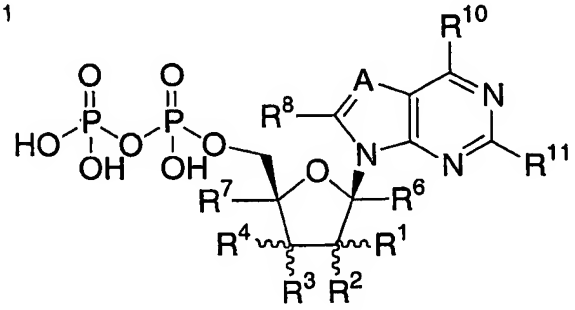


(III)

wherein R1-R11 are as defined above. The compounds of the present invention are also intended to include pharmaceutically acceptable salts of the triphosphate ester as well as pharmaceutically acceptable salts of 5'-monophosphate and 5'-diphosphate ester derivatives of the structural formulae IV and V, respectively,

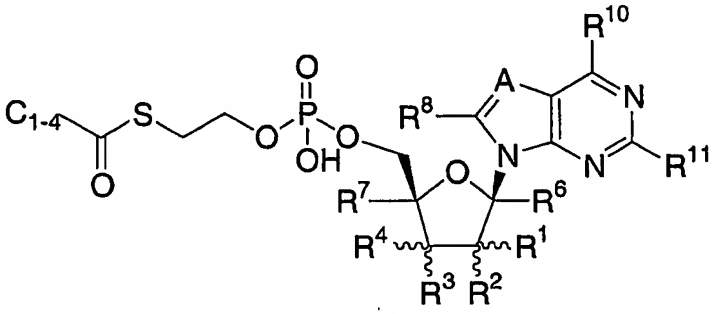


(IV)

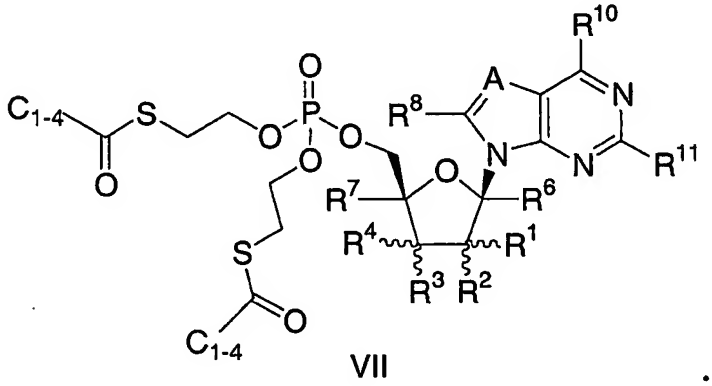


(V)

The term "5'-(S-acyl-2-thioethyl)phosphate" or "SATE" refers to a mono- or di-ester derivative of a 5'-monophosphate nucleoside derivative of the present invention of structural formulae VI and VII, respectively, as well as pharmaceutically acceptable salts of the mono-ester,



VI



VII

The term "composition", as in "pharmaceutical composition," is intended to encompass a product comprising the active ingredient(s) and the inert ingredient(s) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically acceptable carrier.

The terms "administration of" and "administering a" compound should be understood to mean providing a compound of the invention or a prodrug of a compound of the invention to the individual in need.

Another aspect of the present invention is concerned with a method of treating orthopoxvirus infections with a compound of the present invention in combination with one or more agents useful for treating orthopoxvirus infections. Such agents active against orthopoxviruses include, but are not limited to, cidofovir, ribavirin, levovirin, and viramidine. Levovirin is the L-enantiomer of ribavirin which has shown immunomodulatory activity similar to ribavirin. Viramidine represents an analog of ribavirin disclosed in WO 01/60379 (assigned to ICN Pharmaceuticals). In accordance with this method of the present invention, the individual components of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment, and the term "administering" is to be interpreted accordingly. It will be understood that the scope of combinations of the compounds of this invention with other agents useful for treating orthopoxvirus infection includes in principle any combination with any pharmaceutical composition for treating orthopoxvirus infection. When a compound of the present invention or a pharmaceutically acceptable salt thereof is used in combination with a second therapeutic agent active against orthopoxvirus, the dose of each compound may be either the same as or different from the dose when the compound is used alone.

Ribavirin, levovirin, and viramidine may exert their anti-orthopoxvirus effects by modulating intracellular pools of guanine nucleotides via inhibition of the intracellular enzyme inosine monophosphate dehydrogenase (IMPDH). IMPDH is the rate-limiting enzyme on the biosynthetic route in *de novo* guanine nucleotide biosynthesis. Ribavirin is readily phosphorylated intracellularly and the monophosphate derivative is an inhibitor of IMPDH. Thus, inhibition of IMPDH represents another useful target for the discovery of inhibitors of orthopoxvirus replication. Therefore, the compounds of the present invention may also be administered in combination with an inhibitor of IMPDH, such as VX-497, which is disclosed in WO 97/41211 and WO 01/00622 (assigned to Vertex); another IMPDH inhibitor, such as that disclosed in WO 00/25780 (assigned to Bristol-Myers Squibb); or mycophenolate mofetil [see A.C. Allison and E.M. Eugui, Agents Action, 44 (Suppl.): 165 (1993)].

By "pharmaceutically acceptable" is meant that the carrier, diluent, or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Also included within the present invention are pharmaceutical compositions comprising the nucleoside compounds and derivatives thereof of the present invention in association with a pharmaceutically acceptable carrier. Another example of the invention is a pharmaceutical composition made by combining any of the compounds described above and a pharmaceutically acceptable carrier. Another illustration of the invention is a process for making a pharmaceutical composition comprising combining any of the compounds described above and a pharmaceutically acceptable carrier.

Also included within the present invention are pharmaceutical compositions useful for inhibiting orthopoxvirus replication comprising an effective amount of a compound of the present invention and a pharmaceutically acceptable carrier. Pharmaceutical compositions useful for treating orthopoxvirus infection are also encompassed by the present invention. Additionally, the present invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of a compound of the present invention in combination with a therapeutically effective amount of another agent active against orthopoxvirus. Agents active against orthopoxvirus include, but are not limited to, cidofovir, ribavirin, levovirin, and viramidine.

Another aspect of the present invention provides for the use of the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for the manufacture of a medicament for the inhibition of orthopoxvirus replication, in particular vaccinia virus, variola virus replication, cowpox virus replication, and monkeypox virus replication and/or the treatment of orthopoxvirus infection, in particular vaccinia virus, variola virus infection, cowpox virus infection, and monkeypox virus infection. Yet a further aspect of the present invention provides for the nucleoside compounds and derivatives thereof and their pharmaceutical compositions for use as a medicament for the inhibition of orthopoxvirus replication, in particular vaccinia, variola, cowpox, and monkeypox virus replication, and/or for the treatment of orthopoxvirus infection, in particular vaccinia, variola, cowpox, and monkeypox virus infection.

The pharmaceutical compositions of the present invention comprise a compound of structural formula I as an active ingredient or a pharmaceutically

acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

In practical use, the compounds of structural formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally as, for example, liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a

lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Compounds of structural formula I may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

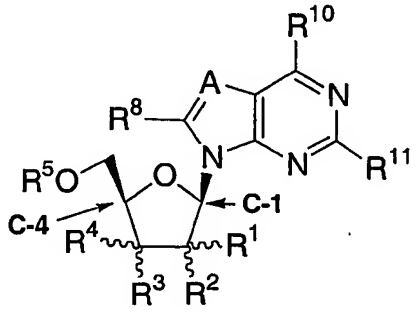
Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of structural formula I are administered orally.

For oral administration to humans, the dosage range is 0.01 to 1000 mg/kg body weight in divided doses. In one embodiment the dosage range is 0.1 to 100 mg/kg body weight in divided doses. In another embodiment the dosage range is 0.5 to 20 mg/kg body weight in divided doses. For oral administration, the compositions are preferably provided in the form of tablets or capsules containing 1.0 to 1000 milligrams of the active ingredient, particularly, 1, 5, 10, 15, 20, 25, 50, 75,

100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, and 1000 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art. This dosage regimen may be adjusted to provide the optimal therapeutic response.

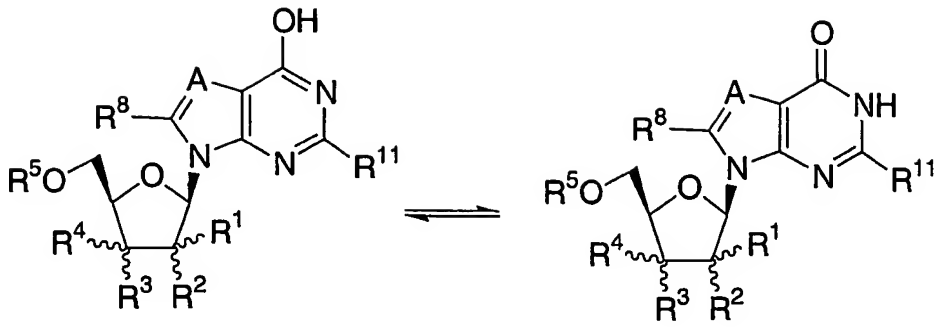
The compounds of the present invention contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. The present invention is meant to comprehend nucleoside compounds having the β-D stereochemical configuration for the five-membered furanose ring as depicted in the structural formula below, that is, nucleoside compounds in which the substituents at C-1 and C-4 of the five-membered furanose ring have the β-stereochemical configuration ("up" orientation as denoted by a bold line).



β-D-

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

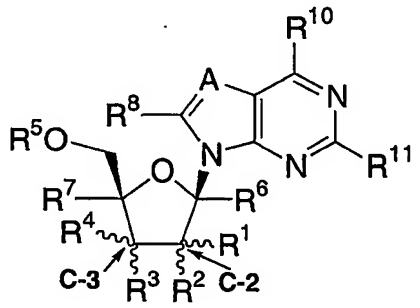
Some of the compounds described herein may exist as tautomers such as keto-enol tautomers. The individual tautomers as well as mixtures thereof are encompassed with compounds of structural formula I. An example of keto-enol tautomers which are intended to be encompassed within the compounds of the present invention is illustrated below:



Compounds of structural formula I may be separated into their individual diastereoisomers by, for example, fractional crystallization from a suitable solvent, for example methanol or ethyl acetate or a mixture thereof, or via chiral chromatography using an optically active stationary phase.

Alternatively, any stereoisomer of a compound of the structural formula I may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known configuration.

The stereochemistry of the substituents at the C-2 and C-3 positions of the furanose ring of the compounds of the present invention of structural formula I is denoted by squiggly lines which signifies that substituents R^1, R^2, R^3 and R^4 can have either the α (substituent "down") or β (substituent "up") configuration independently of one another. Notation of stereochemistry by a bold line as at C-1 and C-4 of the furanose ring signifies that the substituent has the β-configuration (substituent "up").



(I)

The compounds of the present invention may be administered in the form of a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts of

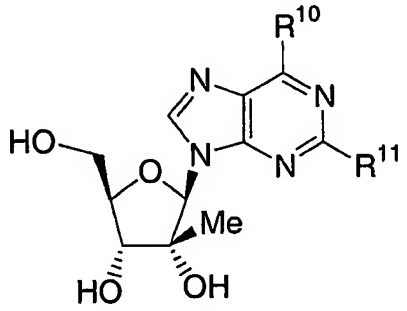
basic compounds encompassed within the term "pharmaceutically acceptable salt" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts of basic compounds of the present invention include, but are not limited to, the following: acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide and valerate. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof include, but are not limited to, salts derived from inorganic bases including aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, mangamous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, cyclic amines, and basic ion-exchange resins, such as arginine, betaine, caffeine, choline, N,N-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

Also, in the case of a carboxylic acid (-COOH) or alcohol group being present in the compounds of the present invention, pharmaceutically acceptable esters of carboxylic acid derivatives, such as methyl, ethyl, or pivaloyloxymethyl, or acyl derivatives of alcohols, such as acetate or maleate, can be employed. Included are those esters and acyl groups known in the art for modifying the solubility or hydrolysis characteristics for use as sustained-release or prodrug formulations.

Preparation of the Nucleoside Compounds and Derivatives of the Invention

The nucleoside compounds and derivatives thereof of the present invention can be prepared following synthetic methodologies well-established in the practice of nucleoside and nucleotide chemistry. Reference is made to the following text for a description of synthetic methods used in the preparation of the compounds of the present invention: "Chemistry of Nucleosides and Nucleotides," L.B. Townsend, ed., Vols. 1-3, Plenum Press, 1988, which is incorporated by reference herein in its entirety.

The synthesis of 9-(2'-C-methyl-β-D-ribofuranosyl)purines of structural formula VIII is described in U.S. Patent No. 3,480,613, the contents of which are incorporated herein in their entirety.

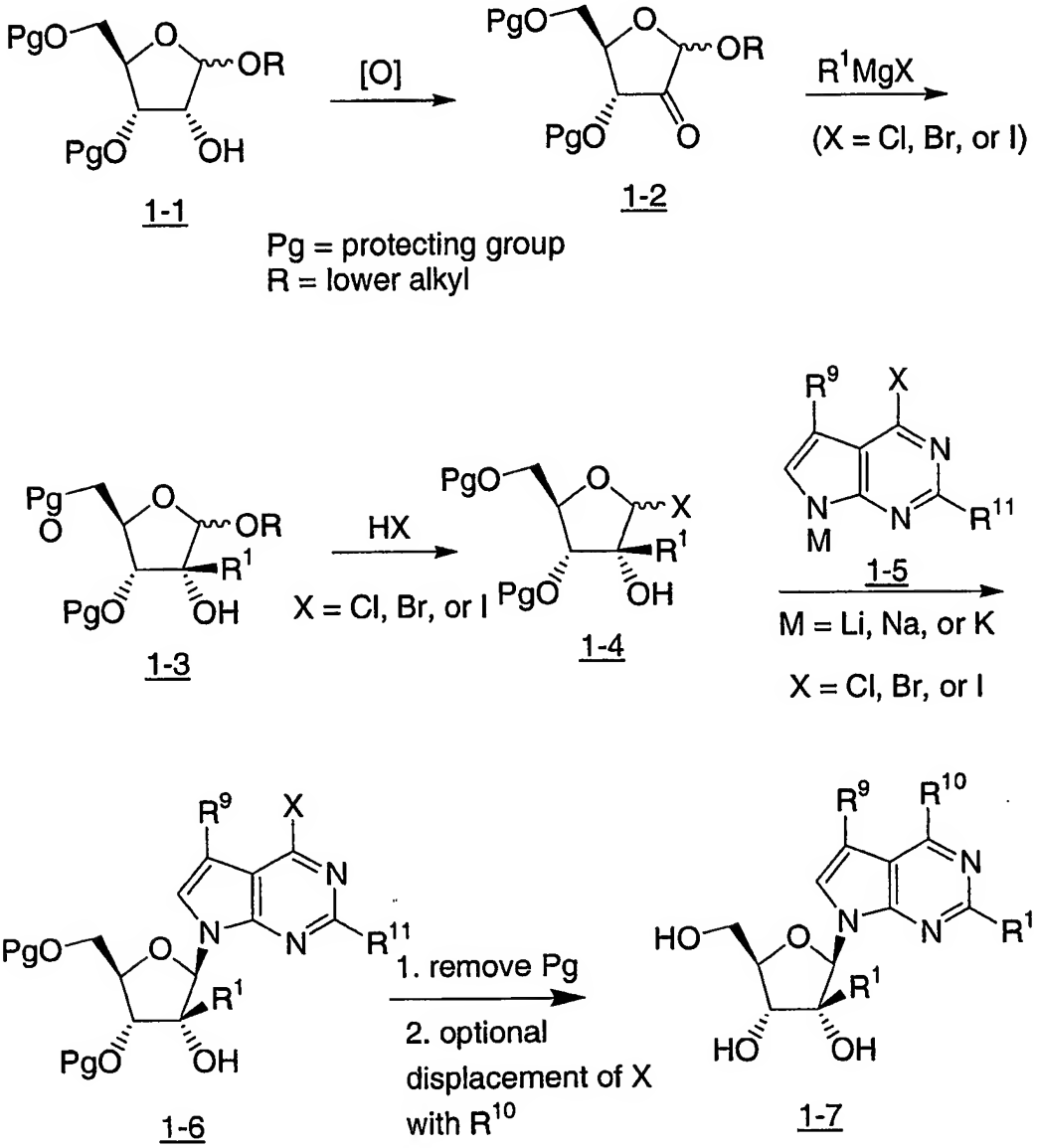


(VIII)

A representative general method for the preparation of compounds of the present invention is outlined in Scheme 1 below. This scheme illustrates the synthesis of compounds of the present invention of structural formula 1-7 wherein the furanose ring has the β-D-ribo configuration. The starting material is a 3,5-bis-O-protected alkyl furanoside, such as methyl furanoside, of structural formula 1-1. The C-2 hydroxyl group is then oxidized with a suitable oxidizing agent, such as a chromium trioxide or chromate reagent, Dess-Martin periodinane, or by Swern oxidation, to afford a C-2 ketone of structural formula 1-2. Addition of a Grignard reagent, such as an alkyl, alkenyl, or alkynyl magnesium halide (for example, MeMgBr, EtMgBr, vinylMgBr, allylMgBr, and ethynylMgBr) or an alkyl, alkenyl, or alkynyl lithium, such as MeLi, across the carbonyl double bond of 1-2 in a suitable organic solvent, such as tetrahydrofuran, diethyl ether, and the like, affords the C-2 tertiary alcohol of structural formula 1-3. A good leaving group (such as Cl, Br, and I) is next introduced at the C-1 (anomeric) position of the furanose sugar derivative by

treatment of the furanoside of formula 1-3 with a hydrogen halide in a suitable organic solvent, such as hydrogen bromide in acetic acid, to afford the intermediate furanosyl halide 1-4. A C-1 sulfonate, such methanesulfonate ($MeSO_2O$-), trifluoromethanesulfonate (CF_3SO_2O-), or p-toluenesulfonate (-OTs), may also serve as a useful leaving group in the subsequent reaction to generate the glycosidic (nucleosidic) linkage. The nucleosidic linkage is constructed by treatment of the intermediate of structural formula 1-4 with the metal salt (such as lithium, sodium, or potassium) of an appropriately substituted 1*H*-pyrrolo[2,3-d]pyrimidine 1-5, such as an appropriately substituted 4-halo-1*H*-pyrrolo[2,3-d]pyrimidine, which can be generated *in situ* by treatment with an alkali hydride (such as sodium hydride), an alkali hydroxide (such as potassium hydroxide), an alkali carbonate (such as potassium carbonate), or an alkali hexamethyldisilazide (such as NaHMDS) in a suitable anhydrous organic solvent, such as acetonitrile, tetrahydrofuran, 1-methyl-2-pyrrolidinone, or N,N-dimethylformamide (DMF). The displacement reaction can be catalyzed by using a phase-transfer catalyst, such as TDA-1 or triethylbenzylammonium chloride, in a two-phase system (solid-liquid or liquid-liquid). The optional protecting groups in the protected nucleoside of structural formula 1-6 are then cleaved following established deprotection methodologies, such as those described in T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons, 1999. Optional introduction of an amino group at the 4-position of the pyrrolo[2,3-d]pyrimidine nucleus is effected by treatment of the 4-halo intermediate 1-6 with the appropriate amine, such as alcoholic ammonia or liquid ammonia, to generate a primary amine at the C-4 position ($-NH_2$), an alkylamine to generate a secondary amine (-NHR), or a dialkylamine to generate a tertiary amine (-NRR'). A 7H-pyrrolo[2,3-d]pyrimidin-4(3H)one compound may be derived by hydrolysis of 1-6 with aqueous base, such as aqueous sodium hydroxide. Alcoholysis (such as methanolysis) of 1-6 affords a C-4 alkoxide (-OR), whereas treatment with an alkyl mercaptide affords a C-4 alkylthio (-SR) derivative. Subsequent chemical manipulations well-known to practitioners of ordinary skill in the art of organic/medicinal chemistry may be required to attain the desired compounds of the present invention.

Scheme 1

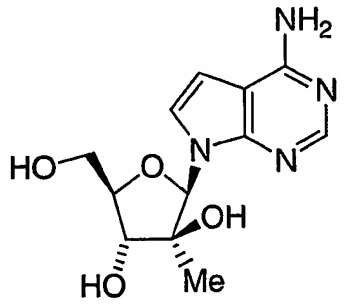


The examples below provide citations to literature publications, which contain details for the preparation of final compounds or intermediates employed in the preparation of final compounds of the present invention. The nucleoside compounds of the present invention were prepared according to procedures detailed in the following examples. The examples are not intended to be limitations on the scope of the instant invention in any way, and they should not be so construed. Those skilled in the art of nucleoside and nucleotide synthesis will readily appreciate that

known variations of the conditions and processes of the following preparative procedures can be used to prepare these and other compounds of the present invention. All temperatures are degrees Celsius unless otherwise noted.

EXAMPLE 1

4-Amino-7-(2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

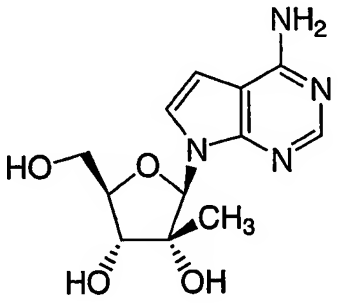


To chromium trioxide (1.57 g, 1.57 mmol) in dichloromethane (DCM) (10 mL) at 0°C was added acetic anhydride (145 mg, 1.41 mmol) and then pyridine (245 mg, 3.10 mmol). The mixture was stirred for 15 min, then a solution of 7-[3,5-*O*-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl]-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [for preparation, see J. Am. Chem. Soc. 105: 4059 (1983)] (508 mg, 1.00 mmol) in DCM (3 mL) was added. The resulting solution was stirred for 2 h and then poured into ethyl acetate (10 mL), and subsequently filtered through silica gel using ethyl acetate as the eluent. The combined filtrates were evaporated in vacuo, taken up in diethyl ether/THF (1:1) (20 mL), cooled to -78°C and methylmagnesium bromide (3M, in THF) (3.30 mL, 10 mmol) was added dropwise. The mixture was stirred at -78°C for 10 min, then allowed to come to room temperature (rt) and quenched by addition of saturated aqueous ammonium chloride (10 mL) and extracted with DCM (20 mL). The organic phase was evaporated in vacuo and the crude product purified on silica gel using 5% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated *in vacuo*. The resulting oil was taken up in THF (5 mL) and tetrabutylammonium fluoride (TBAF) on silica (1.1 mmol/g on silica) (156 mg) was added. The mixture was stirred at room temperature for 30 min, filtered, and evaporated *in vacuo*. The crude product was purified on silica gel using 10%

methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired compound (49 mg) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 1.08 (s, 3H), 3.67 (m, 2H), 3.74 (m, 1H), 3.83 (m, 1H), 5.19 (m, 1H), 5.23 (m, 1H), 5.48 (m, 1H), 6.08 (1H, s), 6.50 (m, 1H), 6.93 (bs, 2H), 7.33 (m, 1H), 8.02 (s, 1H).

EXAMPLE 2

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose

A mixture of 2-*O*-acetyl-3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-ribofuranose [for preparation, see: Helv. Chim. Acta 78: 486 (1995)] (52.4 g, 0.10 mol) in methanolic K_2CO_3 (500 mL, saturated at room temperature) was stirred at room temperature for 45 min and then concentrated under reduced pressure. The oily residue was suspended in CH_2Cl_2 (500 mL), washed with water (300 mL + 5 × 200 mL) and brine (200 mL), dried (Na_2SO_4), filtered, and concentrated to give the title compound (49.0 g) as colorless oil, which was used without further purification in Step B below.

^{1}H NMR (DMSO-d_6): δ 3.28 (s, 3H, OCH_3), 3.53 (d, 2H, $J_{5,4}$ = 4.5 Hz, H-5a, H-5b), 3.72 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,2}$ = 6.6 Hz, H-3), 3.99 (ddd, 1H, $J_{2,1}$ = 4.5 Hz, $J_{2,OH\text{-}2}$ = 9.6 Hz, H-2), 4.07 (m, 1H, H-4), 4.50 (s, 2H, CH_2Ph), 4.52, 4.60 (2d, 2H, J_{gem} = 13.6 Hz, CH_2Ph), 4.54 (d, 1H, OH-2), 4.75 (d, 1H, H-1), 7.32-7.45, 7.52-7.57 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.40, 69.05, 69.74, 71.29, 72.02, 78.41, 81.45, 103.44, 127.83, 127.95, 129.05, 129.28, 131.27, 131.30, 133.22, 133.26, 133.55, 133.67, 135.45, 135.92.

Step B: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose

To an ice-cold suspension of Dess-Martin periodinane (50.0 g, 118 mmol) in anhydrous CH_2Cl_2 (350 mL) under argon (Ar) was added a solution of the compound from Step A (36.2 g, 75 mmol) in anhydrous CH_2Cl_2 (200 mL) dropwise over 0.5 h. The reaction mixture was stirred at 0°C for 0.5 h and then at room temperature for 3 days. The mixture was diluted with anhydrous Et_2O (600 mL) and poured into an ice-cold mixture of $Na_2S_2O_3.5H_2O$ (180 g) in saturated aqueous $NaHCO_3$ (1400 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (600 mL), water (800 mL) and brine (600 mL), dried ($MgSO_4$), filtered and evaporated to give the title compound (34.2 g) as a colorless oil, which was used without further purification in Step C below.

^1H NMR ($CDCl_3$): δ 3.50 (s, 3H, OCH_3), 3.79 (dd, 1H, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 3.5 Hz, H-5a), 3.94 (dd, 1H, $J_{5b,4}$ = 2.3 Hz, H-5b), 4.20 (dd, 1H, $J_{3,1}$ = 1.3 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.37 (ddd, 1H, H-4), 4.58, 4.69 (2d, 2H, J_{gem} = 13.0 Hz, CH_2Ph), 4.87 (d, 1H, H-1), 4.78, 5.03 (2d, 2H, J_{gem} = 12.5 Hz, CH_2Ph), 7.19-7.26, 7.31-7.42 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.72, 69.41, 69.81, 69.98, 77.49, 78.00, 98.54, 127.99, 128.06, 129.33, 129.38, 131.36, 131.72, 133.61, 133.63, 133.85, 133.97, 134.72, 135.32, 208.21.

Step C: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose

To a solution of MeMgBr in anhydrous Et_2O (0.48 M, 300 mL) at –55 °C was added dropwise a solution of the compound from Step B (17.40 g, 36.2 mmol) in anhydrous Et_2O (125 mL). The reaction mixture was allowed to warm to –30°C and stirred for 7 h at –30°C to –15°C, then poured into ice-cold water (500 mL) and the mixture vigorously stirred at room temperature for 0.5 h. The mixture was filtered through a Celite pad (10 × 5 cm) which was thoroughly washed with Et_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in hexanes (~30 mL), applied onto a silica gel column (10 × 7 cm, prepacked in hexanes) and eluted with hexanes and hexanes/EtOAc (9/1) to give the title compound (16.7 g) as a colorless syrup.

^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, $J_{Me,OH}$ = 0.9 Hz, 2C-Me), 3.33 (q, 1H, OH), 3.41 (d, 1H, $J_{3,4}$ = 3.3 Hz), 3.46 (s, 3H, OCH_3), 3.66 (d, 2H, $J_{5,4}$ = 3.7 Hz, H-5a, H-5b), 4.18 (apparent q, 1H, H-4), 4.52 (s, 1H, H-1), 4.60 (s, 2H, C*H*$_2$Ph), 4.63, 4.81 (2d, 2H, J_{gem} = 13.2 Hz, C*H*$_2$Ph), 7.19-7.26, 7.34-7.43 (2m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 24.88, 55.45, 69.95, 70.24, 70.88, 77.06, 82.18, 83.01, 107.63, 127.32, 129.36, 130.01, 130.32, 133.68, 133.78, 134.13, 134.18, 134.45, 134.58.

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step C (9.42 g, 19 mmol) in anhydrous dichloromethane (285 mL) at 0°C was added HBr (5.7 M in acetic acid, 20 mL, 114 mmol) dropwise. The resulting solution was stirred at 0°C for 1 h and then at room temperature for 3 h, evaporated *in vacuo* and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (50 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Chem. Soc., 131 (1960)] (8.76 g, 57 mmol) in anhydrous acetonitrile (1000 mL), and NaH (60% in mineral oil, 2.28 g, 57 mmol), after 4 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h, and then evaporated to dryness. The residue was suspended in water (250 mL) and extracted with EtOAc (2 × 500 mL). The combined extracts were washed with brine (300 mL, dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (10 cm × 10 cm) using ethyl acetate/hexane (1:3 and 1:2) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (5.05 g) as a colorless foam.

^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, CH_3), 3.09 (s, 1H, OH), 3.78 (dd, 1H, $J_{5',5''}$ = 10.9 Hz, $J_{5',4}$ = 2.5 Hz, H-5'), 3.99 (dd, 1H, $J_{5'',4}$ = 2.2 Hz, H-5''), 4.23-4.34 (m, 2H, H-3', H-4'), 4.63, 4.70 (2d, 2H, J_{gem} = 12.7 Hz, C*H*$_2$Ph), 4.71, 4.80 (2d, 2H, J_{gem} = 12.1 Hz,C*H*$_2$Ph), 6.54 (d, 1H, , $J_{5,6}$ = 3.8 Hz, H-5), 7.23-7.44 (m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 21.31, 69.10, 70.41, 70.77, 79.56, 80.41, 81.05, 91.11, 100.57, 118.21, 127.04, 127.46, 127.57, 129.73, 129.77, 130.57, 130.99, 133.51, 133.99, 134.33, 134.38, 134.74, 135.21, 151.07, 151.15 152.47.

Step E: 4-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step D (5.42 g, 8.8 mmol) in dichloromethane (175 mL) at -78°C was added boron trichloride (1M in dichloromethane, 88 mL, 88 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (90 mL) and the resulting mixture stirred at –15°C for 30 min, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 250 mL). The combined filtrate was evaporated, and the residue was purified by flash chromatography over silica gel using CH_2Cl_2 and CH_2Cl_2:MeOH (99:1, 98:2, 95:5 and 90:10) gradient as the eluent to furnish desired compound (1.73 g) as a colorless foam, which turned into an amorphous solid after treatment with MeCN.

^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.61-3.71 (m, 1H, H-5'), 3.79-3.88 (m, 1H, H-5"), 3.89-4.01 (m, 2H, H-3', H-4'), 5.15-5.23 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.24 (s, 1H, H-1'), 6.72 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5), 8.13 (d, 1H, H-6), 8.65 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.20, 59.95, 72.29, 79.37, 83.16, 91.53, 100.17, 117.63, 128.86, 151.13, 151.19, 151.45.

Step F: 4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step E (1.54 g, 5.1 mmol) was added methanolic ammonia (saturated at 0°C; 150 mL). The mixture was heated in a stainless steel autoclave at 85°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column with CH_2Cl_2/MeOH (9/1) as eluent to give the title compound as a colorless foam (0.8 g), which separated as an amorphous solid after treatment with MeCN. The amorphous solid was recrystallized from methanol/acetonitrile; m.p. 222°C.

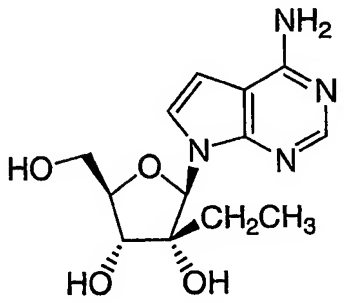
^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H, CH_3), 3.57-3.67 (m, 1H, H-5'), 3.75-3.97 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 5.04 (d, 1H, $J_{3'OH,3'}$ = 6.8 Hz, 3'-OH), 5.06 (t, 1H, $J_{5'OH,5',5"}$ = 5.1 Hz, 5'-OH), 6.11 (s, 1H, H-1'), 6.54 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 6.97 (br s, 2H, NH_2), 7.44 (d, 1H, H-6), 8.02 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.26, 60.42, 72.72, 79.30, 82.75, 91.20, 100.13, 103.08, 121.96, 150.37, 152.33, 158.15.

LC-MS: Found: 279.10 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4+H^+$: 279.11.

EXAMPLE 3

4-Amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-ethyl-1-*O*-methyl-α-D-ribofuranose

To diethyl ether (300 mL) at –78°C was slowly added EtMgBr (3.0 M, 16.6 mL) and then dropwise the compound from Step B of Example 2 (4.80 g, 10.0 mmol) in anhydrous Et_2O (100 mL). The reaction mixture was stirred at –78 °C for 15 min, allowed to warm to –15°C and stirred for another 2 h, and then poured into a stirred mixture of water (300 mL) and Et_2O (600 mL). The organic phase was separated, dried ($MgSO_4$), and evaporated *in vacuo*. The crude product was purified on silica gel using ethyl acetate/hexane (1:2) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (3.87 g) as a colorless oil.

Step B: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-ethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step A (1.02 mg, 2.0 mmol) in dichloromethane (40 mL) was added HBr (5.7 M in acetic acid) (1.75 mL, 10.0 mmol) dropwise at 0°C. The resulting solution was stirred at room temperature for 2 h, evaporated *in vacuo* and co-evaporated twice from toluene (10 mL). The oily residue was dissolved in acetonitrile (10 mL) and added to a vigorously stirred mixture of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (307 mg, 2.0 mmol), potassium hydroxide (337 mg, 6.0 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (130 mg, 0.4 mmol) in acetonitrile (10 mL). The resulting mixture was stirred at room temperature

overnight, and then poured into a stirred mixture of saturated ammonium chloride (100 mL) and ethyl acetate (100 mL). The organic layer was separated, washed with brine (100 mL), dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was purified on silica gel using ethyl acetate/hexane (1:2) as eluent to give the desired product (307 mg) as a colorless foam.

Step C: 4-Chloro-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step B (307 mg, 0.45 mmol) in dichloromethane (8 mL) was added boron trichloride (1M in dichloromethane) (4.50 mL, 4.50 mmol) at -78°C. The mixture was stirred at -78°C for 1h, then at -10°C for 3h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (10 mL), stirred at -15°C for 30 min, and neutralized by addition of aqueous ammonium hydroxide. The mixture was evaporated under diminished pressure and the resulting oil purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (112 mg) as a colorless foam.

Step D: 4-Amino-7-(2-*C*-ethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

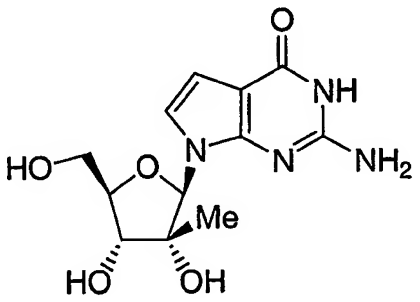
To the compound from Step C (50 mg, 0.16 mmol) was added saturated ammonia in methanol (4 mL). The mixture was stirred at 75°C for 72 h in a closed container, cooled and evaporated *in vacuo*. The crude mixture was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated *in vacuo* to give the desired product (29 mg) as a colorless powder.

^{1}HNMR (200 MHz, DMSO-d_6): δ 0.52 (t, 3H), 1.02 (m, 2H), 4.01-3.24 (m, 6H), 5.06 (m, 1H), 6.01 (s, 1H), 6.51 (d, 1H), 6.95 (s br, 2H), 6.70 (d, 1H), 7.99 (s, 1H).

LC-MS: Found: 295.2 ($M+H^+$); calc. for $C_{13}H_{18}N_4O_4+H^+$: 295.14.

EXAMPLE 4

2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one



Step A: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.27 g, 2.57 mmol) in CH_2Cl_2 (30 mL) was added HBr (5.7 M in acetic acid; 3 mL) dropwise. The reaction mixture was stirred at room temperature for 2 h, concentrated under diminished pressure and coevaporated with toluene (2 × 15 mL). The resulting oil was dissolved in acetonitrile (MeCN) (15 mL) and added dropwise into a well-stirred mixture of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see Heterocycles 35: 825 (1993)] (433 mg, 2.57 mmol), KOH (85%, powdered) (0.51 g, 7.7 mmol), tris-[2-(2-methoxyethoxy)ethyl]amine (165 µL, 0.51 mmol) in acetonitrile (30 mL). The resulting mixture was stirred at room temperature for 1h, filtered and evaporated. The residue was purified on a silica gel column using hexanes/EtOAc, 5/1, 3/1 and 2/1, as eluent to give the title compound as a colorless foam (0.65 g).

Step B: 2-Amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the product from Step A (630 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) at -78°C was added boron trichloride (1M in CH_2Cl_2) (10 mL, 10 mmol). The mixture was stirred at -78°C for 2 h, then at -20°C for 2.5 h. The reaction was quenched with CH_2Cl_2/MeOH (1:1) (10 mL), stirred at -20°C for 0.5 h, and neutralized at 0°C with aqueous ammonia. The solid was filtered, washed with CH_2Cl_2/MeOH (1:1) and the combined filtrate evaporated *in vacuo*. The residue was

purified on a silica gel column with CH_2Cl_2/MeOH, 50/1 and 20/1, as eluent to give the title compound as a colorless foam (250 mg).

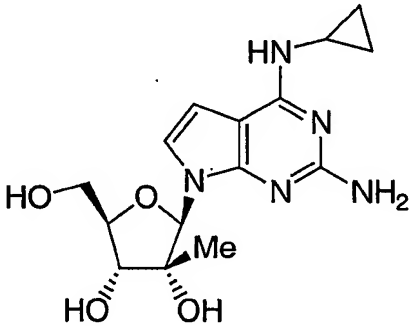
Step C: 2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

A mixture of the product from Step B (90 mg, 0.3 mmol) in aqueous NaOH (2N, 9 mL) was heated at reflux temperature for 5 h, then neutralized at 0°C with 2 N aqueous HCl and evaporated to dryness. Purification on a silica gel column with CH_2Cl_2/MeOH, 5/1, as eluent afforded the title compound as a white solid (70 mg).

^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (s, 3H), 3.79 (m 1H), 3.90-4.05 (m, 3H), 6.06 (s, 1H), 6.42 (d, J = 3.7 Hz, 1H), 7.05 (d, J = 3.7 Hz, 1H).

EXAMPLE 5

2-Amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

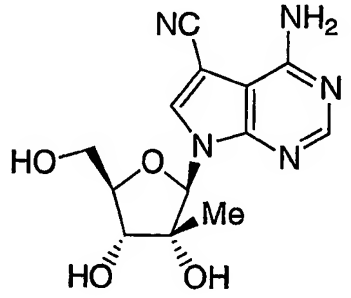


A solution of 2-amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Example 4, Step B) (21 mg, 0.07 mmol) in cyclopropylamine (0.5 mL) was heated at 70°C for two days, then evaporated to an oily residue and purified on a silica gel column with CH_2Cl_2/MeOH, 20/1, as eluent to give the title compound as a white solid (17 mg).

^{1}H NMR (200 MHz, CD_3CN): δ 0.61 (m, 2H), 0.81 (m, 2H), 0.85 (s, 3H), 2.83 (m, 1H), 3.74-3.86 (m, 1H), 3.93-4.03 (m, 2H), 4.11 (d, J = 8.9 Hz, 1H), 6.02 (s, 1H), 6.49 (d, J = 3.7 Hz, 1H), 7.00 (d, J = 3.7 Hz, 1H).

EXAMPLE 6

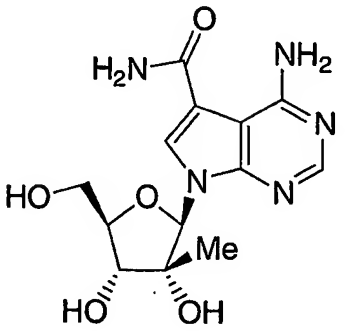
4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile



This compound was prepared following procedures described by Y. Murai et al. in Heterocycles 33: 391-404 (1992).

EXAMPLE 7

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide



This compound was prepared following procedures described by Y. Murai et al. in Heterocycles 33: 391-404 (1992).

EXAMPLE 8

General process to SATE prodrug moiety

S-Acyl-2-thioethyl (SATE) pronucleotides are discussed in C.R. Wagner, V.V. Iyer, and E.J. McIntee, "Pronucleotides: Toward the In Vivo Delivery of Antiviral and Anticancer Nucleotides," Med. Res. Rev., 20: 1-35 (2000), which is

incorporated by reference herein in its entirety. SATE derivatives of nucleosides are also disclosed U.S. Patent Nos. 5,770,725; 5,849,905; and 6,020,482, the contents of each of which are incorporated by reference herein in their entirety.

Bis(*S*-acetyl-2-thioethyl)-*N*,*N*-diisopropylphosphoramidite

2-Mercaptoethanol (5 g, 64 mmol) was dissolved in CH_2Cl_2 (50 mL). To this solution was added triethylamine (7.67 mL, 57.6 mmol), and the reaction mixture was cooled in an ice bath to 0 °C. Acetic anhydride (4.54 mL, 48 mmol) was added dropwise in 10 min, and the reaction mixture was stirred for 1 h at 0 °C. The reaction mixture was then allowed to come to room temperature over a period of 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (75 mL), 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oil. The oil was then dissolved in anhydrous THF (40 mL) and anhydrous triethylamine (7.76 mL) was added. To this mixture was added activated molecular sieves (4Å) and was kept at room temperature for 10 min. The reaction mixture was cooled in an ice bath to 0°C and diisopropylphosphoramidous dichloride (6.47 g, 32.03 mmol) was added. The reaction mixture was stirred at 0 °C for 2 h under inert atmosphere. Hexane (40 mL) was added to the reaction mixture and the precipitate formed was filtered. The filtrate was concentrated to one fourth of the volume, purified by loaded silica gel column chromatography and eluted with hexane containing 3 % triethylamine and incremental amount of ethyl acetate (0 to 7 %) to give the title compound as an oil (2.36 g). ^{1}H NMR ($CDCl_3$): δ 1.17 (s, 6H), 1.21 (s, 6H), 2.36 (s, 6H), 3.14 (t, J = 6.44 Hz), 3.51-3.84 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 24.47, 24.61, 30.48, 42.85, 43.1, 61.88, 62.23, 195.26; ^{13}P NMR ($CDCl_3$): δ 146.96.

EXAMPLE 9

5'-Triphosphate Derivatives

The nucleoside 5'-triphosphates of the present invention were prepared following general procedures described in *Chem. Rev.* 100: 2047 (2000).

EXAMPLE 10

Purification and Purity Analysis of 5'-Triphosphate Derivatives

The triphosphate derivatives were purified by anion exchange (AX) chromatography using a 30 x 100 mm Mono Q column (Pharmacia) with a buffer system of 50 mM Tris, pH 8. Elution gradients were typically from 40 mM NaCl to 0.8 M NaCl in two column volumes at 6.5 mL/min. Appropriate fractions from anion exchange chromatography were collected and desalted by reverse-phase (RP) chromatography using a Luna C18 250 × 21 mm column (Phenomenex) with a flow rate of 10 mL/min. Elution gradients were generally from 1% to 95% methanol in 14 min at a constant concentration of 5 mM triethylammonium acetate (TEAA).

Mass spectra of the purified triphosphates were determined using on-line HPLC mass spectrometry on a Hewlett-Packard (Palo Alto, CA) MSD 1100. A Phenomenex Luna (C18(2)), 150 × 2 mm, plus 30 x 2 mm guard column, 3-μm particle size was used for RP HPLC. A 0 to 50% linear gradient (15 min) of acetonitrile in 20 mM TEAA (triethylammonium acetate) pH 7 was performed in series with mass spectral detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. The mass range of 150-900 was sampled. Molecular masses were determined using the HP Chemstation analysis package.

The purity of the purified triphosphates was determined by analytical RP and AX HPLC. RP HPLC with a Phenomonex Luna or Jupiter column (250 × 4.6 mm), 5-μm particle size was typically run with a 2-70% acetonitrile gradient in 15 min in 100 mM TEAA, pH 7. AX HPLC was performed on a 1.6 × 5 mm Mono Q column (Pharmacia). Triphosphates were eluted with a gradient of 0 to 0.4 M NaCl at constant concentration of 50 mM Tris, pH 8. The purity of the triphosphates was generally >80%.

EXAMPLE 11

5'-Monophosphate Derivatives

The nucleoside 5'-monophosphates of the present invention were prepared following the general procedures described in *Tetrahedron Lett.* 50: 5065 (1967).

EXAMPLE 12

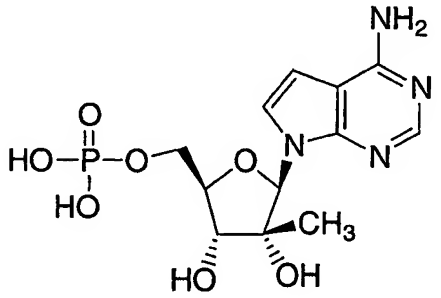
Mass Spectral Characterization of 5'-Triphosphate Derivatives

Mass spectra of 5'-triphosphates of the compounds of the present invention were determined as described in Example 10. Listed in the following table are the calculated and experimental masses for representative 5'-triphosphates prepared according to the procedures of Example 9. The example numbers correspond to the parent compound of the 5'-triphosphate.

Example	Calculated	Found
1	520.0	519.9
2	520.0	520.0
3	534.0	534.0
4	536.0	536.0

EXAMPLE 13

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-monophosphate

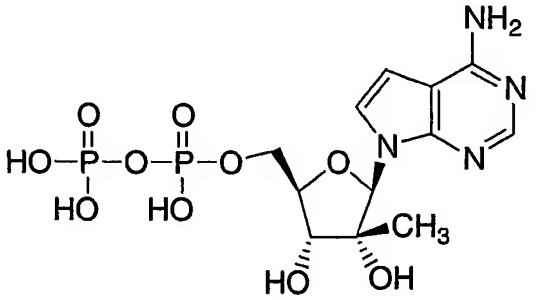


To the compound from Step F of Example 2 (14 mg, 0.05 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (0.5 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.0070 mL, 0.075 mmol) was added via a syringe. The mixture was stirred for 3 h at 0°C, then the reaction was quenched by addition of tetraethylammonium bicarbonate (TEAB) (1M)

(0.5 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.
Electron spray mass spectrum (ES-MS): Found: 359.2 ($M-H^+$), calc. for $C_{12}H_{17}N_4O_7P - H^+$: 359.1.

EXAMPLE 14

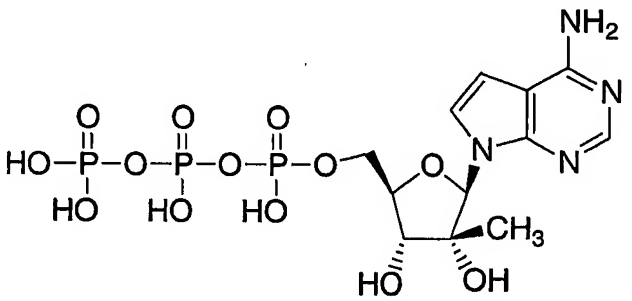
[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-diphosphate



To the compound from Step F of Example 2 (56 mg, 0.20 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (stored over sieves) (1.0 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.023 mL, 0.25 mmol) was added via a syringe. The mixture was stirred for 2 h at 0°C, then tributylamine (0.238 mL, 1.00 mmol) and tributylammonium phosphate (generated from phosphoric acid and tributylamine in pyridine, followed by repeated azeotropic evaporation with pyridine and acetonitrile) (1.0 mmol in 3.30 mL acetonitrile) was added. The mixture was stirred for an additional 30 min at 0°C, the sealed vial was then opened and the reaction quenched by addition of TEAB (1M) (1.0 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.
ES-MS: Found: 439.0 ($M-H^+$), calc. for $C_{12}H_{18}N_4O_{10}P_2 - H^+$: 439.04.

EXAMPLE 15

[4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine]-5'-triphosphate

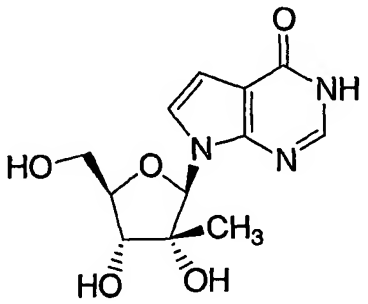


To the compound from Step F of Example 2 (20 mg, 0.07 mmol) (dried by coevaporation with pyridine and several times with toluene) was added trimethyl phosphate (stored over sieves) (0.4 mL). The mixture was stirred overnight in a sealed container. It was then cooled to 0°C and phosphorous oxychloride (0.0070 mL, 0.075 mmol) was added via syringe. The mixture was stirred for 3 h at 0°C, then tributylamine (0.083 mL, 0.35 mmol), tributylammonium pyrophosphate (127 mg, 0.35 mmol) and acetonitrile (stored over sieves) (0.25 mL) were added. The mixture was stirred for an additional 30 min at 0°C, the sealed vial was then opened and the reaction quenched by addition of TEAB (1M) (0.5 mL) and water (5 mL). The reaction mixture was purified and analyzed according to the procedure described in Example 10.

ES-MS: Found: 519.0 ($M-H^+$), calc. for $C_{12}H_{19}N_4O_{13}P_3$- H^+: 519.01.

EXAMPLE 16

7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

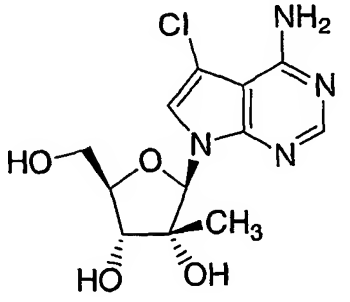


To the compound from Step E of Example 2 (59 mg, 0.18 mmol) was added aqueous sodium hydroxide (1M). The mixture was heated to reflux for 1hr, cooled, neutralized with aqueous HCl (2M) and evaporated in vacuo. The residue was purified on silica gel using dichloromethane/methanol (4:1) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (53 mg) as a colorless oil.

^{1}H NMR (CD_3CN): δ 0.70 (s, 3H), 3.34-4.15 (overlapping m, 7H), 6.16 (s, 1H), 6.57 (d, 3.6 Hz, 1H), 7.37 (d, 3.6 Hz, 1H), 8.83 (s, 1H).

EXAMPLE 17

4-Amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



To a pre-cooled solution (0°C) of the compound from Step F of Example 2 (140 mg, 0.50 mmol) in DMF (2.5 mL) was added *N*-chlorosuccinimide (0.075 g, 0.55 mmol) in DMF (0.5 mL) dropwise. The solution was stirred at room

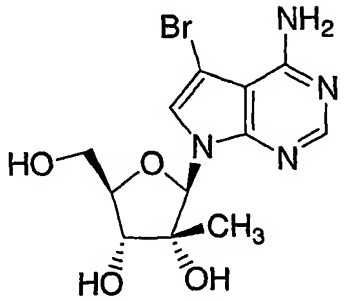
temperature for 1h and the reaction quenched by addition of methanol (4 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (55 mg) as a colorless solid.

^{1}H NMR (CD_3CN): δ 0.80 (s, 3H), 3.65-4.14 (overlapping m, 7H), 5.97 (s br, 2H), 6.17 (s, 1H), 7.51 (s, 1H), 8.16 (s, 1H).

ES-MS: Found: 315.0 ($M+H^+$), calc.for $C_{12}H_{15}ClN_4O_4 + H^+$: 315.09.

EXAMPLE 18

4-Amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



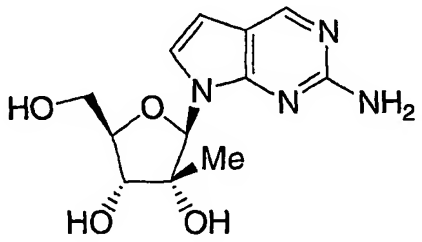
To a pre-cooled solution (0°C) of the compound from Step F of Example 2 (28 mg, 0.10 mmol) in DMF (0.5 mL) was added *N*-bromosuccinimide (0.018 g, 0.10 mmol) in DMF (0.5 mL) dropwise. The solution was stirred at 0°C for 20 min, then at room temperature for 10 min. The reaction was quenched by addition of methanol (4 mL) and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9) as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (13.0 mg) as a colorless solid.

^{1}H NMR (CD_3CN): δ 0.69 (s, 3H), 3.46-4.00 (overlapping m, 7H), 5.83 (s br, 2H), 6.06 (s, 1H), 7.45 (s, 1H), 8.05 (s, 1H).

ES-MS: Found: 359.1 ($M+H^+$), calc.for $C_{12}H_{15}BrN_4O_4 + H^+$: 359.04.

EXAMPLE 19

2-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

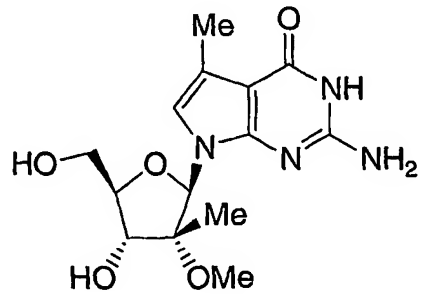


A mixture of 2-amino-4-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (Example 4, Step B) (20 mg, 0.07 mmol) in EtOH (1.0 mL), pyridine (0.1 mL) and 10% Pd/C (6 mg) under H_2 (atmospheric pressure) was stirred overnight at room temperature. The mixture was filtered through a Celite pad which was thorougly washed with EtOH. The combined filtrate was evaporated and purified on a silica gel column with CH_2Cl_2/MeOH, 20/1 and 10/1, as eluent to give the title compound as a white solid (16 mg).

^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (s, 3H, 2'C-Me), 3.82 (dd, $J_{5',4'}$ = 3.6 Hz, $J_{5',5''}$ = 12.7 Hz, 1H, H-5'), 3.94-4.03 (m, 2H, H-5', H-4'), 4.10 (d, $J_{3',4'}$ = 8.8 Hz, 1H, H-3'), 6.02 (s, 1H, H-1'), 6.41 (d, $J_{5,6}$ = 3.8 Hz, 1H, H-5), 7.39 (d, 1H, H-6), 8.43 (s, 1H, H-4). ES MS: 281.4 (MH^+).

EXAMPLE 20

2-Amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one



Step A: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.57 g, 3.16 mmol) in CH_2Cl_2 (50 mL) was added HBr (5.7 M in acetic acid; 3.3 mL) dropwise. The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 2 h, concentrated in vacuo and co-evaporated with toluene (2 × 20 mL). The resulting oil was dissolved in MeCN (20 mL) and added dropwise to a solution of the sodium salt of 2-amino-4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 2-amino-4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see Liebigs Ann. Chem. 1984: 708-721] (1.13 g, 6.2 mmol) in anhydrous acetonitrile (150 mL), and NaH (60% in mineral oil, 248 mg, 6.2 mmol), after 2 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with EtOAc (300 + 150 mL). The combined extracts were washed with brine (100 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (5 × 7 cm) using ethyl acetate/hexane (0 to 30% EtOAc in 5% step gradient) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (0.96 g) as a colorless foam.

Step B: 2-Amino-4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold mixture of the product from Step A (475 mg, 0.7 mmol) in THF (7 mL) was added NaH (60% in mineral oil, 29 mg) and stirred at 0 °C for 0.5 h. Then MeI (48 μL) was added and reaction mixture stirred at room temperature for 24 h. The reaction was quenched with MeOH and the mixture evaporated. The crude product was purified on a silica gel column (5 × 3.5 cm) using hexane/ethyl acetate (9/1, 7/1, 5/1 and 3/1) as eluent. Fractions containing the product were combined and evaporated to give the desired compound (200 mg) as a colorless foam.

Step C: 2-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-one

A mixture of the product from Step B (200 mg, 0.3 mmol) in 1,4-dioxane (15 mL) and aqueous NaOH (2N, 15 mL) in a pressure bottle was heated

overnight at 135 °C. The mixture was then cooled to 0 °C, neutralized with 2N aqueous HCl and evaporated to dryness. The crude product was suspended in MeOH, filtered, and the solid thoroughly washed with MeOH. The combined filtrate was concentrated, and the residue purified on a silica gel column (5 × 5 cm) using CH_2Cl_2/MeOH (40/1, 30/1 and 20/1) as eluent to give the desired compound (150 mg) as a colorless foam.

Step D: 2-Amino-5-methyl-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one

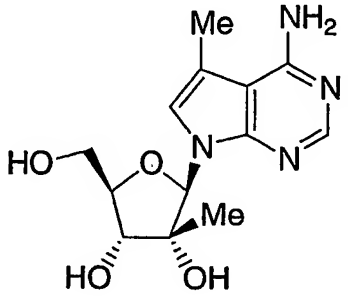
A mixture of the product from Step C (64 mg, 0.1 mmol) in MeOH (5 mL) and Et_3N (0.2 mL) and 10% Pd/C (24 mg) was hydrogenated on a Parr hydrogenator at 50 psi at r.t. for 1.5 days, then filtered through a Celite pad which was thoroughly washed with MeOH. The combined filtrate was evaporated and the residue purified on a silica gel column (3 × 4 cm) with CH_2Cl_2/MeOH (30/1, 20/1) as eluent to yield 2-amino-5-methyl-7-(5-*O*-benzyl-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one. The compound (37 mg) was further hydrogenated in EtOH (2 mL) with 10% Pd/C and under atmospheric pressure of hydrogen. After stirring 2 days at r.t., the reaction mixture was filtered through Celite, the filtrate evaporated and the crude product purified on a silica gel column (1 × 7 cm) with CH_2Cl_2/MeOH (30/1, 20/1 and 10/1) as eluent to yield the title compound (12 mg) after freeze-drying.

^{1}H NMR (200 MHz, CD_3OD): δ 0.81 (s, 3H, 2'C-Me), 2.16 (d, $J_{H\text{-}6,C5\text{-}Me}$ = 1.3 Hz, 3H, C5-Me), 3.41 (s, 3H, 2'-OMe), 3.67 (dd, $J_{5',4'}$= 3.4 Hz, $J_{5',5''}$ = 12.6 Hz, 1H, H-5'), 3.81-3.91 (m, 3H, H-5", H-4', H-3'), 6.10 (s, 1H, H-1'), 6.66 (d, 1H, H-6).

ES MS: 323.3 $(M\text{-}H)^+$.

EXAMPLE 21

4-Amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-methyl-β-D-ribofuranosyl]-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of the product from Step C of Example 2 (1.06 g, 2.1 mmol) in CH_2Cl_2 (30 mL) was added HBr (5.7 M in acetic acid; 2.2 mL) dropwise. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h, concentrated in vacuo and co-evaporated with toluene (2 × 15 mL). The resulting oil was dissolved in MeCN (10 mL) and added dropwise into a solution of the sodium salt of 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine in acetonitrile [generated *in situ* from 4-chloro-5-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Med. Chem. 33: 1984 (1990)] (0.62 g, 3.7 mmol) in anhydrous acetonitrile (70 mL), and NaH (60% in mineral oil, 148 mg, 3.7 mmol), after 2 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was suspended in water (100 mL) and extracted with EtOAc (250 + 100 mL). The combined extracts were washed with brine (50 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (5 × 5 cm) using hexane/ethyl acetate (9/1, 5/1, 3/1) gradient as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (0.87 g) as a colorless foam.

Step B: 4-Chloro-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

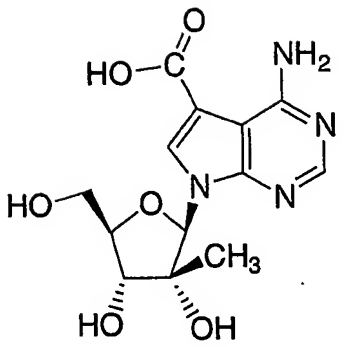
To a solution of the compound from Step A (0.87 g, 0.9 mmol) in dichloromethane (30 mL) at -78°C was added boron trichloride (1M in dichloromethane, 9.0 mL, 9.0 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (9 mL) and the resulting mixture stirred at –15°C for 30 min, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 50 mL). The combined filtrate was evaporated, and the residue was purified on a silica gel column (5 × 5 cm) using CH_2Cl_2 and CH_2Cl_2/MeOH (40/1 and 30/1) gradient as the eluent to furnish the desired compound (0.22 g) as a colorless foam.

Step C: 4-Amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step B (0.2 g, 0.64 mmol) was added methanolic ammonia (saturated at 0°C; 40 mL). The mixture was heated in a stainless steel autoclave at 100°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column (5 × 5 cm) with CH_2Cl_2/MeOH (50/1, 30/1, 20/1) gradient as eluent to give the title compound as a white solid (0.12 g). ^{1}H NMR (DMSO-d_6): δ 0.60 (s, 3H, 2'C-Me), 2.26 (s, 3H, 5C-Me), 3.52-3.61 (m, 1H, H-5'), 3.70-3.88 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 4.91-4.99 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.04 (s, 1H, H-1'), 6.48 (br s, 2H, NH_2), 7.12 (s, 1H, H-6), 7.94 (s, 1H, H-2). ES MS: 295.2 (MH^+).

EXAMPLE 22

4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid

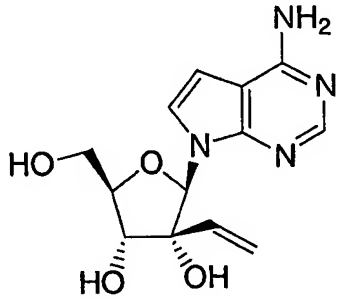


The compound of Example 6 (0.035 g, 0.11 mmol) was dissolved in a mixture of aqueous ammonia (4 mL, 30 wt %) and saturated methanolic ammonia (2 mL), and a solution of H_2O_2 in water (2 mL, 35 wt %) was added. The reaction mixture was stirred at room temperature for 18 h. Solvent was removed under reduced pressure, and the residue obtained was purified by HPLC on a reverse phase column (Altech Altima C-18, 10x 299 mm, A = water, B = acetonitrile, 10 to 60 % B in 50 min, flow 2 mL/min) to yield the title compound (0.015 g, 41 %) as a white solid.

^{1}H NMR (CD_3OD): δ 0.85 (s, 3H, Me), 3.61 (m, 1H), 3.82 (m, 1H) 3.99-4.86 (m, 2H), 6.26 (s, 1H), 8.10 (s, 2H) 8.22(s, 1H); ^{13}C NMR (CD_3OD): 20.13, 61.37, 73.79, 80.42, 84.01, 93.00, 102.66, 112.07, 130.07, 151.40, 152.74, 159.12, 169.30. HRMS (FAB) Calcd for $C_{13}H_{17}N_4O_6^+$ 325.1148, found 325.1143.

EXAMPLE 23

4-Amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-1-*O*-methyl-α-D-ribofuranose

Cerium chloride heptahydrate (50 g, 134.2 mmol) was finely crushed in a pre-heated mortar and transferred to a round-bottom flask equipped with a mechanical stirrer. The flask was heated under high vacuum overnight at 160°C. The vacuum was released under argon and the flask was cooled to room temperature. Anhydrous THF (300 mL) was cannulated into the flask. The resulting suspension was stirred at room temperature for 4 h and then cooled to –78 °C. Vinylmagnesium bromide (1M in THF, 120 mL, 120 mmol) was added and stirring continued at –78 °C for 2 h. To this suspension was added a solution of 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-α-D-erythro-pentofuranose-2-ulose (14 g, 30 mmol) [from Example 2, Step B] in anhydrous THF (100 mL), dropwise with constant stirring. The reaction was stirred at –78 °C for 4 h. The reaction was quenched with saturated ammonium chloride solution and allowed to come to room temperature. The mixture was filtered through a celite pad and the residue washed with Et_2O (2 × 500 mL). The organic layer was separated and the aqueous layer extracted with Et_2O (2 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to a viscous yellow oil. The oil was purified by flash chromatography (SiO_2, 10% EtOAc in hexanes). The title compound (6.7 g, 13.2 mmol) was obtained as a pale yellow oil.

Step B: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Step A (6.4 g, 12.6 mmol) in anhydrous dichloromethane (150 mL) at –20 °C was added HBr (30% solution in AcOH, 20 mL, 75.6 mmol) dropwise. The resulting solution was stirred between –10°C and 0°C for 4 h, evaporated in vacuo and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (100 mL) and added to a solution of the sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (5.8 g, 37.8 mmol) in acetonitrile (generated in situ as described in Example 2) at –20 °C. The resulting mixture was allowed to come to room temperature and stirred at room temperature for 24 h. The mixture was then evaporated to dryness, taken up in water and extracted with EtOAc (2 × 300 mL). The combined extracts were dried over Na_2SO_4, filtered and evaporated. The crude mixture was purified by flash chromatography (SiO_2, 10% EtOAc in hexanes) and the title compound (1.75 g) isolated as a white foam.

Step C: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-vinyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step B (80, mg) was dissolved in the minimum amount of 1,4-dioxane and placed in a stainless steel bomb. The bomb was cooled to –78°C and liquid ammonia was added. The bomb was sealed and heated at 90°C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step D: 4-Amino-7-(2-*C*-vinyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

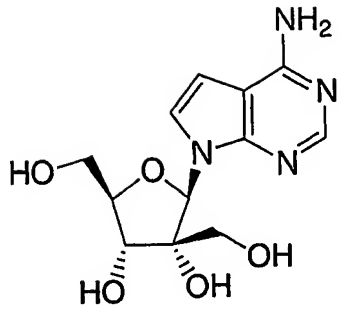
To a solution of the compound from Step C (60 mg) in dichloromethane at –78 °C was added boron trichloride (1M in dichloromethane) dropwise. The mixture was stirred at –78 °C for 2.5 h, then at –30 °C to –20 °C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) and the resulting mixture stirred at –15 °C for 0.5 h, then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with methanol/dichloromethane (1:1). The combined filtrate was evaporated and the residue purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine). The fractions containing the product were evaporated to give the title compound as a white solid (10 mg).

^{1}H NMR (DMSO-d_6): δ 3.6 (m, 1H, H-5'), 3.8 (m, 1H, H-5"), 3.9 (m d, 1-H, H-4'), 4.3 (t, 1H, H-3'), 4.8-5.3(m, 6H, CH=CH_2, 2'-OH, 3'-OH, 5'-OH) 6.12 (s, 1H, H-1'), 6.59 (d, 1H, H-5), 7.1 (br s, 1H, NH2), 7.43 (d, 1H, H-6), 8.01 (s, 1H, H-2).

ES-MS: Found: 291.1 ($M-H^-$); calc. for $C_{13}H_{16}N_4O_4$ - H^-: 291.2.

EXAMPLE 24

4-Amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-hydroxymethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Example 23, Step B (300 mg, 0.48 mmol) in 1,4-dioxane (5 mL) were added *N*-methylmorpholine-*N*-oxide (300 mg, 2.56 mmol) and osmium tetroxide (4% solution in water, 0.3 mL). The mixture was stirred in the dark for 14 h. The precipitate was removed by filtration through a celite plug, diluted with water (3 ×), and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated in vacuo. The oily residue was taken up in dichloromethane (5 mL) and stirred over $NaIO_4$ on silica gel (3 g, 10% $NaIO_4$) for 12 h. The silica gel was removed by filtration and the residue was evaporated and taken up in absolute ethanol (5 mL). The solution was cooled in an ice bath and sodium borohydride (300 mg, 8 mmol) was added in small portions. The resulting mixture was stirred at room temperature for 4 h and then diluted with EtOAc. The organic layer was washed with water (2 × 20 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to give the title compound (160 mg, 0.25 mmol) as white flakes.

Step B: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-hydroxymethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step A (150 mg, 0.23 mmol) was dissolved in the minimum amount of 1,4-dioxane (10 mL) and placed in a stainless steel bomb. The bomb was cooled to –78 °C and liquid ammonia was added. The bomb was sealed and heated at 90°C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step C: 4-Amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

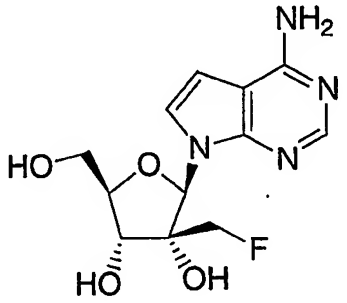
The compound from Step B (120 mg, 0.2 mmol) was dissolved in 1:1 methanol/dichloromethane, 10% Pd-C was added, and the suspension stirred under an H_2 atmosphere for 12 h. The catalyst was removed by filtration through a celite pad and washed with copious amounts of methanol. The combined filtrate was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine) to give the title compound (50 mg) as a white powder.

^1H NMR (CD_3OD): δ 3.12 (d, 1H, CH_2'), 3.33 (d, 1H, CH_2''), 3.82 (m, 1H, H-5'), 3.99-4.1(m, 2H, H-4', H-5"), 4.3 (d, 1H, H-3'), 6.2 (s, 1H, H-1'), 6.58 (d, 1H, H-5), 7.45 (d, 1H, H-6), 8.05 (s, 1H, H-2).

LC-MS: Found: 297.2 ($M+H^+$); calc. for $C_{12}H_{16}N_4O_5 + H^+$: 297.3.

EXAMPLE 25

4-Amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-fluoromethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of the compound from Example 24, Step A (63 mg, 0.1 mmol) in anhydrous dichloromethane (5 mL) under argon, were added 4-dimethylaminopyridine (DMAP) (2 mg, 0.015 mmol) and triethylamine (62 μL, 0.45 mmol). The solution was cooled in an ice bath and p-toluenesulfonyl chloride (30 mg, 0.15 mmol) was added. The reaction was stirred at room temperature overnight, washed with $NaHCO_3$ (2 × 10 mL), water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated to a pink solid in vacuo. The solid was dissolved in anhydrous THF (5 mL) and cooled in an icebath. Tetrabutylammonium fluoride (1M solution in THF, 1 mL, 1 mmol) was added and the mixture stirred at room temperature for 4 h. The solvent was removed in vacuo, the residue taken up in dichloromethane, and washed with $NaHCO_3$ (2 × 10 mL), water (10 mL) and brine (10 mL). The dichloromethane layer was dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to afford the title compound (20 mg) as a white solid.

Step B: 4-Amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*-fluoromethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step A (18 mg, 0.03 mmol) was dissolved in the minimum amount of 1,4-dioxane and placed in a stainless steel bomb. The bomb was cooled to –78 °C and liquid ammonia was added. The bomb was sealed and heated at

90 °C for 24 h. The ammonia was allowed to evaporate and the residue concentrated to a white solid which was used in the next step without further purification.

Step C: 4-Amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The compound from Step B (16 mg) was dissolved in 1:1 methanol/dichloromethane, 10% Pd-C was added, and the suspension stirred under an H_2 atmosphere for 12 h. The catalyst was removed by filtration through a celite pad and washed with copious amounts of methanol. The combined filtrate was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, 10% methanol in EtOAc containing 0.1% triethylamine) to give the title compound (8 mg) as a white powder.

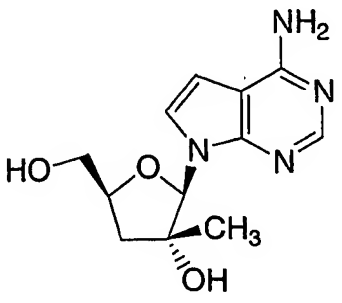
^1H NMR (DMSO-d_6): δ 3.6-3.7 (m, 1H, H-5'), 3.8 – 4.3 (m, 5H, H-5'', H-4', H-3', CH_2) 5.12 (t, 1H, 5'-OH), 5.35 (d, 1H, 3'-OH), 5.48 (s, 1H, 2'-OH), 6.21 (s, 1H, H-1'), 6.52 (d, 1H, H-5), 6.98 (br s, 2H, NH2), 7.44 (d, 1 H, H-6), 8.02 (s, 1H, H-2).

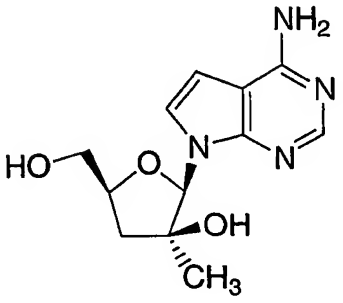
^{19}F NMR (DMSO-d_6): δ -230.2 (t).

ES-MS: Found: 299.1 ($M+H^+$), calc.for $C_{12}H_{15}FN_4O_4 + H^+$: 299.27.

EXAMPLES 26 and 27

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine and 4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine





Step A: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*] pyrimidine

To a stirred solution of tubercidin (5.0 g, 18.7 mmol) in a mixture of pyridine (7.5 mL) and DMF (18.5 mL) was added silver nitrate (6.36 g, 38.8 mmol). This mixture was stirred at room temperature for 2 h. It was cooled in an ice bath and THF (37.4 mL) and *tert*-butyldimethylsilyl chloride (5.6 g, 37 mmol) was added and the mixture was stirred at room temperature for 2 h. The mixture was then filtered through a pad of celite and washed with THF. The filtrate and washings were diluted with ether containing a small amount of chloroform. The organic layer was washed successively with sodium bicarbonate and water (3 × 50 mL), dried over anhydrous sodium sulfate and concentrated. The pyridine was removed by coevaporation with toluene and the residue was purified by flash chromatography on silica gel using 5-7% MeOH in CH_2Cl_2 as the eluent; yield 3.0 g.

Step B: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of mixture of the compounds from Step A (3.0 g, 6.0 mmol) in anhydrous pyridine (30 mL) was added 4,4'-dimethoxytrityl chloride (2.8 g, 8.2 mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was then triturated with aqueous pyridine and extracted with ether. The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated to a yellow foam (5.6 g). The residue was purified by flash chromatography over silica gel using 20-25% EtOAc in hexanes as the eluent. The appropriate fractions were collected and concentrated to furnish 2',5'-bis-O-(*tert*-butyldimethylsilyl)- and 3',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleosides as colorless foams (2.2 g and 1.0 g, respectively).

Step C: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*O*-tosyl-β-D-ribofuranosyl)]-4-[di-(4-methoxyphenyl)phenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cooled solution of 2',5'-bis-O-(*tert*-butyldimethylsilyl)-protected nucleoside from Step B (2.0 g, 2.5 mmol) in pyridine (22 mL) was added p-toluenesulfonyl chloride (1.9 g, 9.8 mmol). The reaction mixture was stirred at room temperature for four days. It was then triturated with aqueous pyridine (50%, 10 mL)

and extracted with ether (3 × 50 mL) containing a small amount of CH_2Cl_2 (10 mL). The organic layer was washed with sodium bicarbonate and water (3 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Pyridine was removed by co-evaporation with toluene (3 × 25 mL). The residual oil was filtered through a pad of silica gel using hexane:ethyl acetate (70:30) as eluent; yield 1.4 g.

Step D: 4-[di-(4-methoxyphenyl)phenylmethyl]amino-7-[3-*O*-tosyl-β-D-ribofuranosyl-7H-pyrrolo[2,3-*d*]pyrimidine

A solution of the compound from Step C (1.0 g, 1.1 mmol) and THF (10 mL) was stirred with tetrabutylammonium fluoride (1M solution in THF, 2.5 mL) for 0.5h. The mixture was cooled and diluted with ether (50 mL). The solution was washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated to an oil. The residue was purified by passing through a pad of silica gel using hexane: ethyl acetate (1:1) as eluent; yield 780 mg.

Step E: 4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]- pyrimidine and 4-amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine

A solution of CH_3MgI (3.0 M solution in ether, 3.0 mL) in anhydrous toluene (3.75 mL) was cooled in an ice bath. To this was added a solution of the compound from Step D (500 mg, 0.8 mmol) in anhydrous toluene (3.7 mL). The resulting mixture was stirred at room temperature for 3.5 h. It was cooled and treated with aqueous NH_4Cl solution and extracted with ether (50 mL containing 10 mL of CH_2Cl_2). The organic layer was separated and washed with brine (2 × 30 mL) and water (2 × 25 mL), dried over anhydrous Na_2SO_4 and concentrated to an oil which was purified by flash chromatography on silica gel using 4% MeOH in CH_2Cl_2 to furnish the 2-C-α-methyl compound (149 mg) and the 2-C-β-methyl compound (34 mg). These derivatives were separately treated with 80% acetic acid and the reaction mixture stirred at room temperature for 2.5 h. The acetic acid was removed by repeated co-evaporation with ethanol and toluene. The residue was partitioned between chloroform and water. The aqueous layer was washed with chloroform and concentrated. The evaporated residue was purified on silica gel using 5-10% MeOH in CH_2Cl_2 as the eluent to furnish the desired compounds as white solids.

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (9.0 mg):

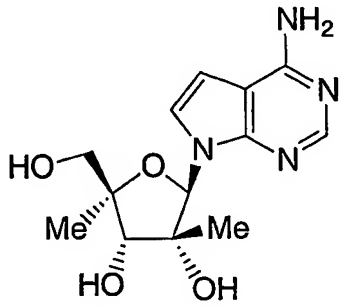
^{1}H NMR (DMSO-d_6): δ 0.74 (s, 3H, CH_3), 1.77 (dd, 1H, H-3'), 2.08 (t, 1H, H-3"), 3.59 (m, 1H, H-5'), 3.73 (m, 1H, H-5"), 4.15 (m, 1H, H-4'), 5.02 (t, 1H, OH-5'), 5.33 (s, 1H, OH-2'), 6.00 (s, 1H, H-1'), 6.54 (d, 1H, H-7), 6.95 (br s, 2H, NH_2), 7.47 (d, 1H, H-8), 8.00 (s, 1H, H-2); ES-MS: 263.1 [M-H].

4-Amino-7-(3-deoxy-2-*C*-methyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (15 mg):

^{1}H NMR (DMSO-d_6): δ 1.23 (s, 3H, CH_3), 2.08 (ddd, 2H, H-3'and 3"), 3.57 (m, 2H, H-5'and 5"), 4.06 (m, 1H, H-4), 5.10 (s, 1H, OH-2'), 5.24 (t, 1H, OH-5'), 6.01 (s, 1H, H-1'), 6.49 (d, 1H, H-7),6.89 (br s, 2H, NH_2), 7.35 (d, 1H, H-8), 8.01 (s,1H,H-2). ES-MS: 265.2[M+H].

EXAMPLE 28

4-Amino-7-(2,4-*C*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 5-Deoxy-1,2-*O*-isopropylidene-D-xylofuranose

1,2-*O*-Isopropylidene-D-xylofuranose (38.4 g, 0.2 mol), 4-dimethylaminopyridine (5 g), triethylamine (55.7 mL, 0.4 mol) were dissolved in dichloromethane (300 mL). p-Toluenesulfonyl chloride (38.13 g, 0.2 mol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then poured into saturated aqueous sodium bicarbonate (500 mL) and the two layers were separated. The organic layer was washed with aqueous citric acid solution (20%, 200 mL), dried (Na_2SO_4) and evaporated to give a solid (70.0 g). The solid was dissolved in dry THF (300 mL) and $LiAlH_4$ (16.0 g, 0.42 mol) was added in portions over 30 min. The mixture was stirred at room temperature for 15. Ethyl

acetate (100 mL) was added dropwise over 30 min and the mixture was filtered through a silica gel bed. The filtrate was concentrated and the resulting oil was chromatographed on silica gel (EtOAc/hexane 1/4) to afford the product as a solid (32.5 g).

Step B: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-1-*O*-methyl-4-methyl-α-D-ribofuranose

Chromium oxide (50 g, 0.5 mol), acetic anhydride (50 mL, 0.53 mol) and pyridine (100 mL, 1.24 mol) were added to dichloromethane (1 L) in an ice-water bath and the mixture was stirred for 15 min. 5-Deoxy-1,2-*O*-isopropylidene-D-xylofuranose (32 g, 0.18 mol) in dichloromethane (200 mL) was added, and the mixture was stirred at the same temperature for 30 min. The reaction solution was diluted with ethyl acetate (1 L) and filtered through a silica gel bed. The filtrate was concentrated to give a yellow oil. The oil was dissolved in 1,4-dioxane (1 L) and formaldehyde (37%, 200 mL). The solution was cooled to 0°C and solid KOH (50 g) was added. The mixture was stirred at room temperature overnight and was then extracted with ethyl acetate (6 × 200 mL). After concentration, the residue was chromatographed on silica gel (EtOAc) to afford the product as an oil (1.5 g). The oil was dissolved in 1-methyl-2-pyrrolidinone (20 mL) and 2,4-dichlorophenylmethyl chloride (4 g, 20.5 mmol) and NaH (60%, 0.8 g) were added. The mixture was stirred overnight and diluted with toluene (100 mL). The mixture was then washed with saturated aqueous sodium bicarbonate (3 × 50 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in methanol (50 mL) and HCl in dioxane (4 M, 2 mL) was added. The solution was stirred overnight and evaporated. The residue was chromatographed on silica gel (EtOAc/hexane:1/4) to afford the desired product as an oil (2.01 g).

Step C: 3,5-Bis-*O*-(2,4-dichlorophenylmethyl)-2,4-di-*C*-methyl-1-*O*-methyl-α-D-ribofuranose

The product (2.0 g, 4.0 mmol) from Step B and Dess-Martin periodinane (2.0 g) in dichloromethane (30 mL) were stirred overnight at room temperature and then concentrated under reduced pressure. The residue was triturated with ether ether (50 mL) and filtered. The filtrate was washed with a solution of $Na_2S_2O_3.5H_2O$ (2.5 g) in saturated aqueous sodium bicarbonate solution (50 mL),

dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhydrous Et_2O (20 mL) and was added dropwise to a solution of MeMgBr in Et_2O (3 M, 10 mL) at –78 °C. The reaction mixture was allowed to warm to –30°C and stirred at –30°C to –15°C for 5 h, then poured into saturated aqueous ammonium chloride (50 mL). The two layers were separated and the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was chromatographed on silica gel (EtOAc/hexane: 1/9) to afford the title compound as a syrup (1.40 g).

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2,4-di-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step C (0.70 g, 1.3 mmol) was added HBr (5.7 M in acetic acid, 2 mL). The resulting solution was stirred at room temperature for 1 h, evaporated in vacuo and co-evaporated with anhydrous toluene (3 × 10 mL). 4-Chloro-*1H*-pyrrolo[2,3-*d*]pyrimidine (0.5 g, 3.3 mmol) and powdered KOH (85%, 150 mg, 2.3 mmol) were stirred in 1-methyl-2-pyrrolidinone (5 mL) for 30 min and the mixture was co-evaporated with toluene (10 mL). The resulting solution was poured into the above bromo sugar residue and the mixture was stirred overnight. The mixture was diluted with toluene (50 mL), washed with water (3 × 50 mL) and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with EtOAc/ Hexane (15/85) to afford a solid (270 mg).

Step E: 4-Amino-7-(2,4-*C*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

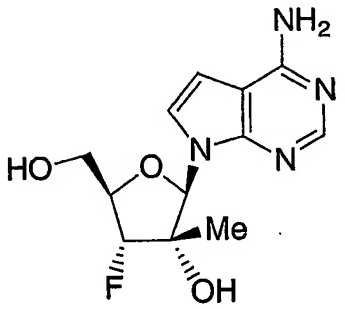
The compound from Step D (270 mg) was dissolved in dioxane (2 mL) and liquid ammonia (20 g) was added in a stainless steel autoclave. The mixture was heated at 100°C for 15, then cooled and evaporated. The residue was chromatographed on silica gel (EtOAc) to afford a solid (200 mg). The solid (150 mg) and Pd/C (10% 150 mg) in methanol (20 mL) were shaken under H_2 (30 psi) for 3 h, filtered and evaporated. The residue was chromatographed on silica gel (MeOH/CH_2Cl_2: 1/9) to afford the desired product as a solid (35 mg).
^1H NMR (DMSO-d_6): δ 0.65 (s, 3H), 1.18 (s, 3H), 3.43 (m, 2H), 4.06 (d, 1H, *J* 6.3 Hz), 4.87 (s, 1H), 5.26 (br, 1H), 5.08 (d, 1H, *J* 6.3 Hz), 5.25 (t, 1H, *J* 3.0 Hz), 6.17 (s, 1H), 6.54 (d, 1H, *J* 3.5 Hz), 6.97 (s, br, 2H), 7.54 (d, 1H, *J* 3.4 Hz), 8.02 (s, 1H).

^{13}C NMR (DMSO-d_6): δ 18.19, 21.32, 65.38, 73.00, 79.33, 84.80, 90.66, 99.09, 102.41, 121.90, 149.58, 151.48, 157.38.

LC-MS: Found: 295.1 ($M+H^+$); calculated for $C_{13}H_{18}N_4O_4+H^+$: 295.1.

EXAMPLE 29

4-Amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 3-Deoxy-3-fluoro-1-*O*-methyl-5-*O*-toluoyl-α-D-ribofuranose

1,2-*O*-Isopropylidene-D-xylofuranose (9.0 g, 50 mmol) and *p*-toluoyl chloride (7.0 mL, 50 mmol) in pyridine (50 mL) were stirred for 30 min. Water (10 mL) was added and the mixture was concentrated under reduced pressure. The residue was dissolved in toluene (500 mL) and the solution was washed with water (200 mL) and saturated aqueous sodium bicarbonate (200 mL). The two layers were separated and the organic layer was evaporated. The residue was dissolved in methanol (100 mL) and HCl in dioxane (4 M, 10 mL) was added. The mixture was stirred at room temperature overnight and was then evaporated under reduced pressure. The resulting oil was chromatographed on silica gel (EtOAc/hexane: 1/1) to afford an oil (10.1 g). The oil was dissolved in dichloromethane (100 mL) and diethylaminosulfur trifluoride (DAST) (5.7 mL) was added. The mixture was stirred overnight and was then poured into saturated aqueous sodium bicarbonate solution (100 mL). The mixture was extracted with toluene (2 × 50 mL) and the combined organic layers were concentrated. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford the title compound as an oil (1.50 g).

Step B: 3-Deoxy-3-fluoro-2-*C*-methyl-1-*O*-methyl-5-*O*-toluoyl-α-D-ribofuranose

The product from Step A (1.0 g, 3.5 mmol) and Dess-Martin periodinane (2.5 g) in dichloromethane (20 mL) were stirred overnight at room temperature and was then concentrated under reduced pressure. The residue was triturated with diethyl ether (50 mL) and filtered. The filtrate was washed with a solution of $Na_2S_2O_3.5H_2O$ (12.5 g) in saturated aqueous sodium bicarbonate (100 mL), dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhydrous THF (50 mL). $TiCl_4$ (3 mL) and methyl magnesium bromide in ethyl ether (3 M, 10 mL) were added at –78°C and the mixture was stirred at –50 to –30°C for 2 h. The mixture was poured into saturated aqueous sodium bicarbonate solution (100 mL) and filtered through Celite. The filtrate was extracted with toluene (100 mL) and evaporated. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford the title compound as an oil (150 mg).

Step C: 4-Amino-7-(3-deoxy-3-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

The product from Step B (150 mg, 0.5 mmol) was dissolved in HBr (30%) in acetic acid (2 mL). After one hour, the mixture was evaporated under reduced pressure and co-evaporated with toluene (10 mL). 4-Chloro-*1H*-pyrrolo[2,3-*d*]pyrimidine (0.5 g, 3.3 mmol) and powdered KOH (85%, 150 mg, 2.3 mmol) were stirred in DMF (3 mL) for 30 min and the mixture was co-evaporated with toluene (2 mL). The resulting solution was poured into the above bromo sugar and the mixture was stirred overnight. The mixture was diluted with toluene (50 mL), washed with water (3 × 50 mL) and concentrated under reduced pressure. The residue was chromatographed on silica gel (EtOAc/hexane: 15/85) to afford an oil (60 mg). The oil was dissolved in dioxane (2 mL) and liquid ammonia (20 g) was added in a stainless steel autoclave. The mixture was heated at 85°C for 18 h, then cooled and evaporated. The residue was chromatographed on silica gel (methanol/dichloromethane: 1/9) to afford the title compound as a solid (29 mg). ^{1}H NMR (DMSO-d_6): δ 0.81 (s, 3H), 3.75 (m, 2H), 4.16 (m, 1H), 5.09 (dd, 1H, *J* 53.2, 7.8 Hz), 5.26 (br, 1H), 5.77 (s, 1H), 6.15 (d, 1H, *J* 2.9 Hz), 6.59 (d, 1H, *J* 3.4 Hz), 7.02 (s br, 2H), 7.39 (d, 1H, *J* 3.4 Hz), 8.06 (s, 1H).

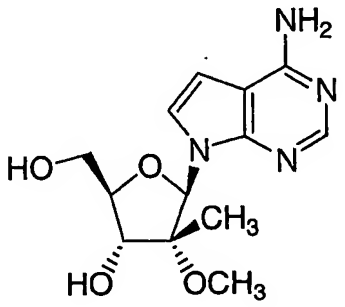
^{13}C NMR (DMSO-d_6): 19.40, 59.56, 77.24, 79.29, 90.15, 91.92, 99.88, 102.39, 121.17, 149.80, 151.77, 157.47.

^{19}F NMR (DMSO-d_6): δ 14.66 (m).

ES-MS: Found: 283.1 ($M+H^+$); calculated for $C_{12}H_{15}FN_4O_3+H^+$: 283.1.

EXAMPLE 30

4-Amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 4-chloro-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a pre-cooled (0°C) solution of the compound from Example 2, Step D (618 mg, 1.0 mmol) in THF (8 mL) was added methyl iodide (709 mg, 5.0 mmol) and NaH (60% in mineral oil) (44 mg, 1.1 mmol). The resulting mixture was stirred overnight at room temperature and then poured into a stirred mixture of saturated aqueous ammonium chloride (50 mL) and dichloromethane (50 mL). The organic layer was washed with water (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The resulting crude product was purified on silica gel using ethyl acetate/hexane as the eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (735 mg) as a colorless foam.

Step B: 4-amino-7-[3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine

To the compound from Step A (735 mg, 1.16 mmol) was added methanolic ammonia (saturated at 0°C) (20 mL). The mixture was heated in a stainless steel autoclave at 80°C overnight, then cooled and the content evaporated in vacuo. The crude mixture was purified on silica gel using ethyl acetate/hexane as the

eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (504 mg) as colorless foam.

Step C: 4-amino-7-(2-*C*,2-*O*-dimethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

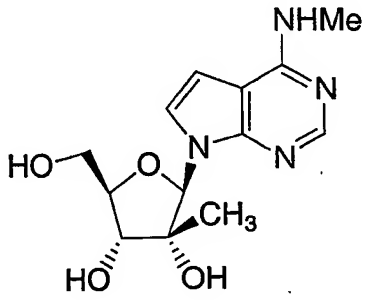
A mixture of the product from Step C (64 mg, 0.1 mmol), MeOH (5 mL), Et_3N (0.2 mL) and 10% Pd/C (61 mg) was hydrogenated on a Parr hydrogenator at 50 psi at room temperature overnight. The mixture was filtered throught celite, evaporated in vacuo and filtered through a pad of silica gel using 2% methanol in dichloromethane as eluent. The desired product was collected and evaporated in vacuo. The compound was redissolved in methanol (10 mL) and 10% Pd/C (61 mg) was added. The mixture was hydrogenated on a Parr hydrogenator at 55 psi at room temperature for two weeks. The mixture was filtered through celite, evaporated in vacuo and purified on silica gel using 10% methanol in dichloromethane as eluent. Fractions containing the product were pooled and evaporated in vacuo to give the desired product (110 mg) as a colorless foam.

^1H NMR (DMSO-d_6): δ 0.68 (s, 3H,), 3.40 (s, 3H), 3.52-3.99 (overlapping m, 4H), 4.92 (d, 1H), 5.07 (t, 1H), 6.26 (s, 1H), 6.55 (d, 1H), 7.00s br, 2H), 7.46 (d, 1H), 8.05 (s, 1H).

LC-MS: Found: 293.1 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4-H^+$: 293.12.

EXAMPLE 31

4-Methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

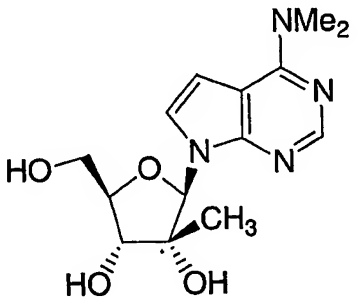


The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to methylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture

was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (144 mg).
^{1}H NMR (DMSO-d_6): δ 0.63 (s, 3H, CH_3), 3.32 (s, 3H, N CH_3), 3.58-3.67 (m, 1H, H-5'), 3.79-3.39 (m, 3H, H-5", H-4', H-3'), 5.03 (s, 1H, 2'-OH), 5.04-5.11 (1H,3'-OH, 1H, 5'-OH), 6.14 (s, 1H, H-1'), 6.58 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.46 (d, 1H, H-6), 7.70 (br s, 1H, NH), 8.14 (s, 1H, H-2).
LC-MS: Found: 295.1 (M-H^+); calc. for $C_{13}H_{18}N_4O_4$+H^+: 294.3.

EXAMPLE 32

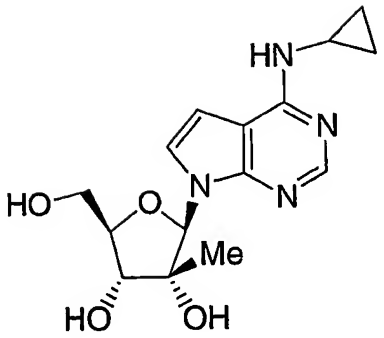
4-Dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to dimethylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (164 mg).
^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.29 (s, 3H, N CH_3), 3.32 (s, 3H, N CH_3), 3.60-3.66 (m, 1H, H-5'), 3.77-3.97 (m, 3H, H-5", H-4', H-3'), 5.04 (s, 1H, 2'-OH), 5.06-5.11 (1H, 3'-OH, 1H, 5'-OH), 6.21 (s, 1H, H-1'), 6.69 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.55 (d, 1H, H-6), 8.13 (s, 1H, H-2).
LC-MS: Found: 309.3 (M-H^+); calc. for $C_{14}H_{20}N_4O_4$+H^+: 308.33.

EXAMPLE 33

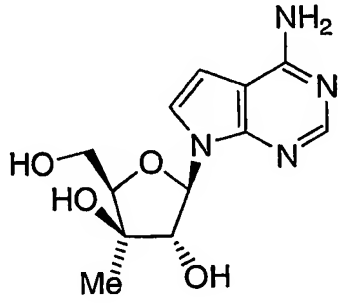
4-Cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



The compound from Step E of Example 2 (200 mg, 0.67 mmol) was added to cyclopropylamine (5 mL condensed in a small stainless steel autoclave) and warmed at 85°C for 48 h, then cooled and evaporated in vacuo. The crude mixture was purified on a silica gel with ethanol as the eluent to give the title compound which separated as an amorphous solid after treatment with MeCN. The amorphous solid was dissolved in water and lyophilized to give a colorless powder (148 mg).
^{1}H NMR (DMSO-d_6): δ 0.51- 0.58 (m, 2H), 0.64 (s, 3H, CH_3), 0.74- 0.076 (m, 2H), 3.62-3.67 (m, 1H, H-5'), 3.79-3.82 (m, 3H, H-5"), 3.92-3.96 (m, H-4', H-3'), 5.03 (s, 1H, 2'-OH), 5.05-5.10 (1H, 3'-OH, 1H, 5'-OH), 6.15 (s, 1H, H-1'), 7.48 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 7.59 (d, 1H, H-6), 8.13 (s, 1H, H-2).
LC-MS: Found: 321.1 (M-H$^+$); calc. for $C_{15}H_{20}N_4O_4$+H$^+$: 320.3.

EXAMPLE 34

4-Amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



Step A: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a solution of mixture of the compounds from Step A of Examples 26 and 27 (0.32 g, 0.65 mmol) in anhydrous pyridine (6 mL) was added monomethoxytrityl chloride (0.30 g, 0.98 mmol) and the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated and the residue was partitioned between CH_2Cl_2 (70 mL) and water (20 mL). The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column using 5-13% EtOAc in hexanes as the eluent. The appropriate fractions were collected and concentrated to furnish 2',5'-bis-O-(*tert*-butyldimethylsilyl)- and 3',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleosides as colorless foams (343 mg and 84 mg, respectively).

Step B: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranos-3-ulosyl]-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a well-stirred suspension of chromium trioxide (91 mg, 0.91 mmol) in CH_2Cl_2 (4 mL) at 0°C were added pyridine (147 μL, 1.82 mmol) and then acetic anhydride (86 μL, 0.91 mmol). The mixture was stirred at room temperature for 0.5 h. Then the 2',5'-bis-O-(*tert*-butyldimethylsilyl) protected nucleoside from step A (343 mg 0.45 mmol) in CH_2Cl_2 (2.5 mL) was added and the mixture stirred at room temperature 2 h. The mixture was then poured into ice-cold EtOAc (10 mL) and filtered through a short silica gel column using EtOAc as the eluent. The filtrate was evaporated and the residue purified on a silica gel column with hexanes and hexanes/EtOAc (7/1) as the eluent to give the title compound (180 mg).

Step C: 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*C*-methyl-β-D-ribofuranosyl)-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine and 7-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-*C*-methyl-β-D-xylofuranosyl)-4-[(4-methoxyphenyl)diphenylmethyl]amino-7*H*-pyrrolo[2,3-*d*]pyrimidine

To a mixture of MeMgBr (3.0 M solution in ether; 0.17 mL, 0.5 mmol) in anhydrous hexanes (1.5 mL) at room temperature was added dropwise a solution of the compound from Step B (78 mg, 0.1 mmol) in anhydrous hexanes (0.5 mL). After 2 h stirring at room temperature, the reaction mixture was poured into ice-cold water (10 mL) and diluted with EtOAc (20 mL), then filtered through Celite which was then thoroughly washed with EtOAc. The layers were separated and the organic layer was

washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified on a silica gel column using 8 to 25% EtOAc in hexanes as eluent to give the 3-C-methyl xylo- (60 mg) and the 3-C-methyl ribo-isomer (20 mg).

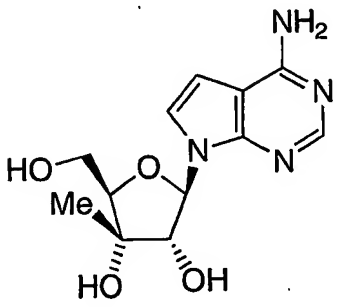
Step D: 4-Amino-7-(3-*C*-methyl-β-D-xylofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

To an ice-cold solution of 3-C-methyl-xylo isomer from Step C (60 mg, 0.08 mmol) in THF (2 mL) was added TBAF (1 M in THF; 0.32 mL, 0.32 mmol). The reaction mixture was stirred at room temperature for 5 h, then diluted with CH_2Cl_2 (50 mL), washed with water (3 × 15 mL), dried,and evaporated. The residue was dissolved in dioxane (0.3 mL) and 80% acetic acid (3 mL) was added. The reaction mixture was stirred at room temperature for 24 h and then evaporated. The residue was co-evaporated with dioxane, taken up in water (50 mL) and washed with CH_2Cl_2 (2 × 10 mL). The aqueous layer was concentrated and then freeze-dried. The residue was purified on silica gel column with CH_2Cl_2/MeOH (20/1 and 10/1) as the eluent to give the title compound as a white fluffy compound after freeze drying (10 mg).

^1H NMR (CD_3CN): δ 1.28 (s, 3H, CH_3), 3.56 (br s, 1H, OH), 3.78 (m, 3H, H-4', H-5', H-5"), 4.10 (br s, 1H, OH), 4.44 (d, 1H, $J_{2'1'}$ = 3.9 Hz, H-2'), 5.58 (d, 1H, H-1'), 5.85 (br s, 2H, NH_2), 6.15 (br s, 1H, OH), 6.48 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5), 7.23 (d, 1H, H-6), 8.11 (s, 1H, H-2). ES-MS: 281 $[MH]^+$.

EXAMPLE 35

4-Amino-7-(3-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine

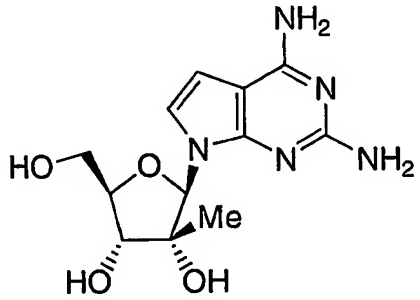


The ribo-isomer (20 mg) from Step C of Example 32 was deprotected using the procedure described in Step D of Example 32 to yield the title compound (4 mg).

^{1}H NMR (CD_3CN): δ 1.43 (s, 3H, CH_3), 3.28 (br s, 1H, OH), 3.58 (m, 2H, H-5', H-5"), 3.99 (m, 1H, H-4'), 4.10 (br s, 1H, OH), 4.62 (d, 1H, $J_{2',1'}$ = 8.1 Hz, H-2'), 5.69 (d, 1H, H-1'), 5.88 (br s, 3H, OH, NH_2), 6.45 (br s, 1H, OH), 6.51 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5), 7.19 (d, 1H, H-6), 8.12 (s, 1H, H-2). ES-MS: 281 $[MH]^+$.

EXAMPLE 36

2,4-Diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



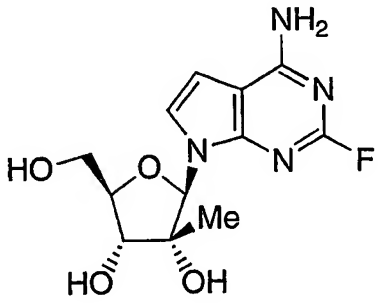
A mixture of the product from Step B of Example 4 (24 mg) in aqueous ammonia (30%, 10 mL) was heated in a stainless steel autoclave at 100 °C overnight, then cooled and evaporated. The residue was purified on a silica gel column with CH_2Cl_2/MeOH (10/1 and 5/1) as the eluent to afford the title compound (15 mg).

^{1}H NMR (DMSO-d_6): δ 0.68 (s, 3H, CH_3), 3.48-3.58 (m 1H, H-5'), 3.68-3.73 (m, 2H, H-5", H-4'), 3.84 (m, 1H, H-3'), 4.72 (s, 1H, 2'-OH), 4.97-5.03 (m, 2H, 3'-OH, 5'-OH), 5.45 (br s, 2H, NH_2), 6.00 (s, 1H, H-1'), 6.28 (d, 1H, J = 3.7 Hz, H-5), 6.44 (br s, 2H, NH_2) 6.92 (d, 1H J = 3.7 Hz, H-6).

ES MS: 294.1 ($M-H^+$).

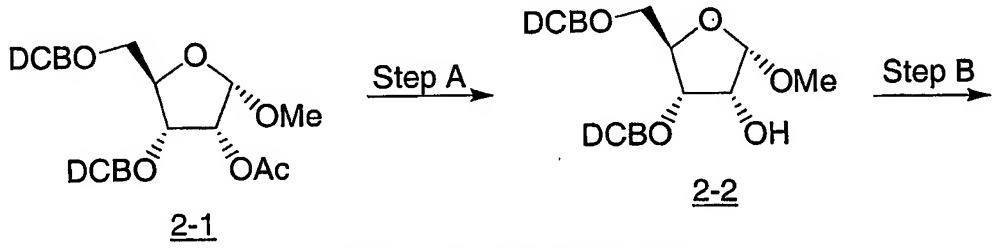
EXAMPLE 37

4-Amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine



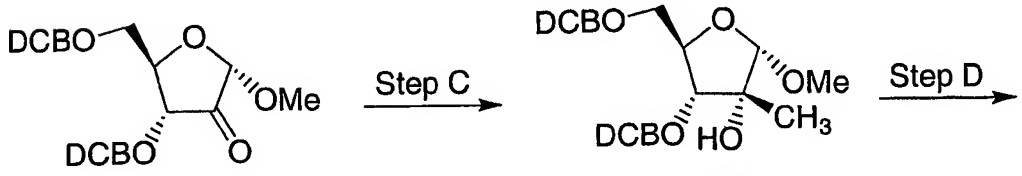
To a solution of HF/pyridine (70%, 2 mL) diluted with pyridine (1 mL) at –30 °C is added the compound of Example 36 (60 mg, 0.2 mmol) in 0.5 mL pyridine followed by *tert*-butyl nitrite (36 μL, 0.3 mmol). Stirring is continued for 5 min at –25 °C. Then the solution is poured into ice-water (5 mL), neutralized with 2 N aqueous NaOH, and evaporated to dryness. The residue is purified on a silica gel column with CH_2Cl_2/MeOH (20/1 and 10/1) as the eluent to afford the title compound.

Scheme 2

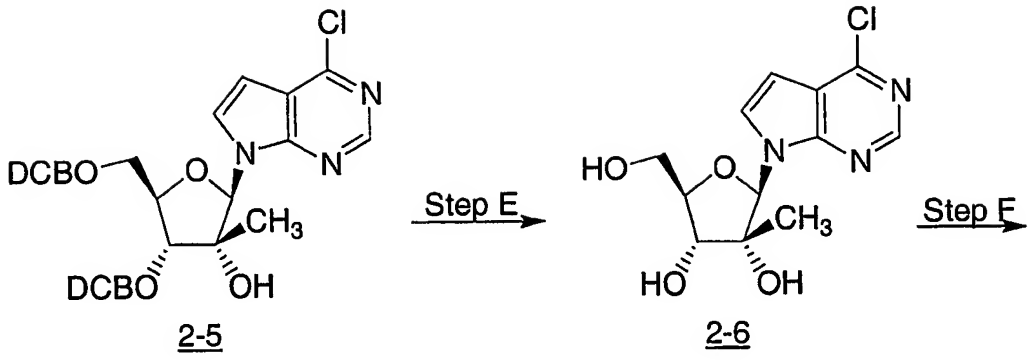


2-1 2-2

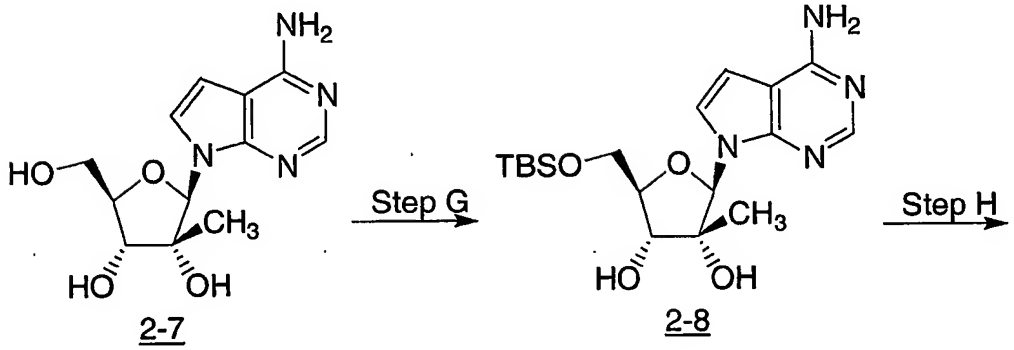
(DCB = 2,4-dichlorobenzyl)



2-3 2-4

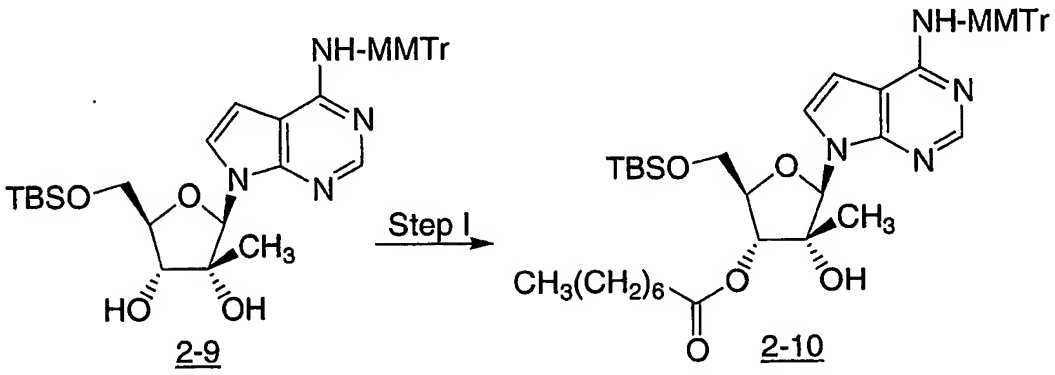


2-5 2-6



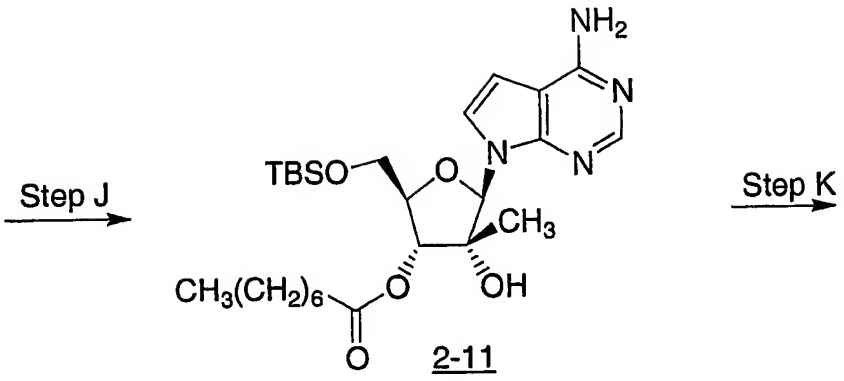
2-7 2-8

(TBS = *tert*-butyldimethylsilyl)

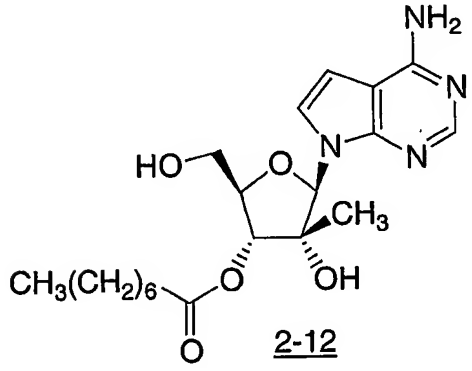


2-9 2-10

(MMTr = p-methoxyphenyldiphenylmethyl)



2-11



2-12

EXAMPLE 38

4-Amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-12)

Step A: 3,5-Bis-*O*-(2,4-dichlorobenzyl)-1-*O*-methyl-α-D-ribofuranose (1-2)

A mixture of 2-*O*-acetyl-3,5-bis-*O*-(2,4-dichlorobenzyl)-1-*O*-methyl-α-D-ribofuranose (2-1) [for preparation, see: Helv. Chim. Acta 78: 486 (1995)] (52.4 g, 0.10 mol) in methanolic K_2CO_3 (500 mL, saturated at room temperature) was stirred at room temperature for 45 min. and then concentrated under reduced pressure. The oily residue was suspended in CH_2Cl_2 (500 mL), washed with water (300 mL + 5 × 200 mL) and brine (200 mL), dried (Na_2SO_4), filtered, and concentrated to give the title compound (49.0 g) as colorless oil, which was used without further purification in Step B below.

^{1}H NMR (DMSO-d_6): δ 3.28 (s, 3H, OCH_3), 3.53 (d, 2H, $J_{5,4}$ = 4.5 Hz, H-5a, H-5b), 3.72 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,2}$ = 6.6 Hz, H-3), 3.99 (ddd, 1H, $J_{2,1}$ = 4.5 Hz, $J_{2,OH-2}$ = 9.6 Hz, H-2), 4.07 (m, 1H, H-4), 4.50 (s, 2H, CH_2Ph), 4.52, 4.60 (2d, 2H, J_{gem} = 13.6

Hz, CH_2Ph), 4.54 (d, 1H, OH-2), 4.75 (d, 1H, H-1), 7.32-7.45, 7.52-7.57 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6) δ 55.40, 69.05, 69.74, 71.29, 72.02, 78.41, 81.45, 103.44, 127.83, 127.95, 129.05, 129.28, 131.27, 131.30, 133.22, 133.26, 133.55, 133.67, 135.45, 135.92.

Step B: 3,5-Bis-*O*-(2,4-dichlorobenzyl)-1-*O*-methyl-α-D-*erythro*-pentofuranos-2-ulose (2-3)

To an ice-cold suspension of Dess-Martin periodinane (50.0 g, 118 mmol) in anhydrous CH_2Cl_2 (350 mL) under argon (Ar) was added a solution of the compound from Step A (36.2 g, 75 mmol) in anhydrous CH_2Cl_2 (200 mL) dropwise over 0.5 h. The reaction mixture was stirred at 0°C for 0.5 h and then at room temperature for 3 days. The mixture was diluted with anhydrous Et_2O (600 mL) and poured into an ice-cold mixture of $Na_2S_2O_3.5H_2O$ (180 g) in saturated aqueous $NaHCO_3$ (1400 mL). The layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (600 mL), water (800 mL) and brine (600 mL), dried ($MgSO_4$), filtered and evaporated to give the title compound (34.2 g) as a colorless oil, which was used without further purification in Step C below.

^{1}H NMR ($CDCl_3$): δ 3.50 (s, 3H, OCH_3), 3.79 (dd, 1H, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 3.5 Hz, H-5a), 3.94 (dd, 1H, $J_{5b,4}$ = 2.3 Hz, H-5b), 4.20 (dd, 1H, $J_{3,1}$ = 1.3 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.37 (ddd, 1H, H-4), 4.58, 4.69 (2d, 2H, J_{gem} = 13.0 Hz, CH_2Ph), 4.87 (d, 1H, H-1), 4.78, 5.03 (2d, 2H, J_{gem} = 12.5 Hz, CH_2Ph), 7.19-7.26, 7.31-7.42 (2m, 10H, 2Ph).

^{13}C NMR (DMSO-d_6): δ 55.72, 69.41, 69.81, 69.98, 77.49, 78.00, 98.54, 127.99, 128.06, 129.33, 129.38, 131.36, 131.72, 133.61, 133.63, 133.85, 133.97, 134.72, 135.32, 208.21.

Step C: 3,5-Bis-*O*-(2,4-dichlorobenzyl)-2-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (2-4)

To a solution of MeMgBr in anhydrous Et_2O (0.48 M, 300 mL) at –55 °C was added dropwise a solution of the compound from Step B (17.40 g, 36.2 mmol) in anhydrous Et_2O (125 mL). The reaction mixture was allowed to warm to –30°C and stirred for 7 h at –30°C to –15°C, then poured into ice-cold water (500 mL) and the mixture vigorously stirred at room temperature for 0.5 h. The mixture was filtered through a Celite pad (10 × 5 cm) which was thoroughly washed with Et_2O.

The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in hexanes (~30 mL), applied onto a silica gel column (10 × 7 cm, prepacked in hexanes) and eluted with hexanes and hexanes/EtOAc (9/1) to give the title compound (16.7 g) as a colorless syrup.

^{1}H NMR ($CDCl_3$): δ 1.36 (d, 3H, $J_{Me,OH}$ = 0.9 Hz, 2C-Me), 3.33 (q, 1H, OH), 3.41 (d, 1H, $J_{3,4}$ = 3.3 Hz), 3.46 (s, 3H, OCH_3), 3.66 (d, 2H, $J_{5,4}$ = 3.7 Hz, H-5a, H-5b), 4.18 (apparent q, 1H, H-4), 4.52 (s, 1H, H-1), 4.60 (s, 2H, C*H*$_2$Ph), 4.63, 4.81 (2d, 2H, J_{gem} = 13.2 Hz, C*H*$_2$Ph), 7.19-7.26, 7.34-7.43 (2m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 24.88, 55.45, 69.95, 70.24, 70.88, 77.06, 82.18, 83.01, 107.63, 127.32, 129.36, 130.01, 130.32, 133.68, 133.78, 134.13, 134.18, 134.45, 134.58.

Step D: 4-Chloro-7-[3,5-bis-*O*-(2,4-dichlorobenzyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-5)

To a solution of the compound from Step C (9.42 g, 19 mmol) in anhydrous dichloromethane (285 mL) at 0°C was added HBr (5.7 M in acetic acid, 20 mL, 114 mmol) dropwise. The resulting solution was stirred at 0°C for 1 h and then at room temperature for 3h, evaporated *in vacuo* and co-evaporated with anhydrous toluene (3 × 40 mL). The oily residue was dissolved in anhydrous acetonitrile (50 mL) and added to a solution of sodium salt of 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine [for preparation, see J. Chem. Soc., 131 (1960)] in acetonitrile [generated *in situ* from 4-chloro-1*H*-pyrrolo[2,3-*d*]pyrimidine (8.76 g, 57 mmol) in anhydrous acetonitrile (1000 mL), and NaH (60% in mineral oil, 2.28 g, 57 mmol), after 4 h of vigorous stirring at room temperature]. The combined mixture was stirred at room temperature for 24 h, and then evaporated to dryness. The residue was suspended in water (250 mL) and extracted with EtOAc (2 × 500 mL). The combined extracts were washed with brine (300 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified on a silica gel column (10 cm × 10 cm) using ethyl acetate/hexane (1:3 and 1:2) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product (5.05 g) as a colorless foam.

^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, CH_3), 3.09 (s, 1H, OH), 3.78 (dd, 1H, $J_{5',5''}$ = 10.9 Hz, $J_{5',4}$ = 2.5 Hz, H-5'), 3.99 (dd, 1H, $J_{5'',4}$ = 2.2 Hz, H-5''), 4.23-4.34 (m, 2H, H-3', H-4'), 4.63, 4.70 (2d, 2H, J_{gem} = 12.7 Hz, C*H*$_2$Ph), 4.71, 4.80 (2d, 2H, J_{gem} = 12.1 Hz,C*H*$_2$Ph), 6.54 (d, 1H, , $J_{5,6}$ = 3.8 Hz, H-5), 7.23-7.44 (m, 10H, 2Ph).

^{13}C NMR ($CDCl_3$): δ 21.31, 69.10, 70.41, 70.77, 79.56, 80.41, 81.05, 91.11, 100.57, 118.21, 127.04, 127.46, 127.57, 129.73, 129.77, 130.57, 130.99, 133.51, 133.99, 134.33, 134.38, 134.74, 135.21, 151.07, 151.15 152.47.

Step E: 4-Chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-6)

To a solution of the compound from Step D (5.42 g, 8.8 mmol) in dichloromethane (175 mL) at -78°C was added boron trichloride (1M in dichloromethane, 88 mL, 88 mmol) dropwise. The mixture was stirred at –78°C for 2.5 h, then at –30°C to –20°C for 3 h. The reaction was quenched by addition of methanol/dichloromethane (1:1) (90 mL) and the resulting mixture stirred at –15°C for 30 min., then neutralized with aqueous ammonia at 0°C and stirred at room temperature for 15 min. The solid was filtered and washed with CH_2Cl_2/MeOH (1/1, 250 mL). The combined filtrate was evaporated, and the residue was purified by flash chromatography over silica gel using CH_2Cl_2 and CH_2Cl_2:MeOH (99:1, 98:2, 95:5 and 90:10) gradient as the eluent to furnish desired compound (1.73 g) as a colorless foam, which turned into an amorphous solid after treatment with MeCN.

^{1}H NMR (DMSO-d_6): δ 0.64 (s, 3H, CH_3), 3.61-3.71 (m, 1H, H-5'), 3.79-3.88 (m, 1H, H-5"), 3.89-4.01 (m, 2H, H-3', H-4'), 5.15-5.23 (m, 3H, 2'-OH, 3'-OH, 5'-OH), 6.24 (s, 1H, H-1'), 6.72 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5), 8.13 (d, 1H, H-6), 8.65 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.20, 59.95, 72.29, 79.37, 83.16, 91.53, 100.17, 117.63, 128.86, 151.13, 151.19, 151.45.

Step F: 4-Amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-7)

To the compound from Step E (1.54 g, 5.1 mmol) was added methanolic ammonia (saturated at 0°C; 150 mL). The mixture was heated in a stainless steel autoclave at 85°C for 14 h, then cooled and evaporated *in vacuo*. The crude mixture was purified on a silica gel column with CH_2Cl_2/MeOH (9/1) as eluent to give the title compound as a colorless foam (0.8 g), which separated as an amorphous solid after treatment with MeCN. The amorphous solid was recrystallized from methanol/acetonitrile; m.p. 222°C.

^{1}H NMR (DMSO-d_6): δ 0.62 (s, 3H, CH_3), 3.57-3.67 (m, 1H, H-5'), 3.75-3.97 (m, 3H, H-5", H-4', H-3'), 5.00 (s, 1H, 2'-OH), 5.04 (d, 1H, $J_{3'OH,3'}$ = 6.8 Hz, 3'-OH), 5.06 (t, 1H, $J_{5'OH,5',5"}$ = 5.1 Hz, 5'-OH), 6.11 (s, 1H, H-1'), 6.54 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5), 6.97 (br s, 2H, NH_2), 7.44 (d, 1H, H-6), 8.02 (s, 1H, H-2).

^{13}C NMR (DMSO-d_6): δ 20.26, 60.42, 72.72, 79.30, 82.75, 91.20, 100.13, 103.08, 121.96, 150.37, 152.33, 158.15.

LC-MS: Found: 279.10 ($M-H^+$); calc. for $C_{12}H_{16}N_4O_4+H^+$: 279.11.

Step G: 4-Amino-7-[5-*O*-(*tert*-butyldimethylsilyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-8)

To a solution of the compound from Step F (457 mg, 1.63 mmol) in anhydrous pyridine (3.5 mL) was added *tert*-butyldimethylsilyl chloride (370 mg, 2.45 mmol). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then diluted with ethyl acetate (40 mL) which was washed with saturated aqueous sodium bicarbonate solution (20 mL). The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and evaporated to an oil that was subjected to chromatography on silica gel eluting with 10% MeOH in CH_2Cl_2. The appropriate fractions were collected, evaporated, and dried under high vacuum to furnish the title compound as a colorless foam (516 mg).

^{1}H NMR (DMSO-d_6): δ 7.95 (s, 1H), 7.35 (d, 1H, J = 3.4Hz), 6.89 (bs, 2H, NH_2), 6.44 (d, 1H, J = 3.4Hz), 6.02 (s, 1H), 5.01-4.98 (m, 2H), 3.92-3.70 (m, 3H), 3.40-3.25 (m, 1H), 0.82 (s, 9H), 0.54 (s, 3H), 0.00 (s, 6H).

Step H: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[5-*O*-(*tert*-butyldimethylsilyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-9)

To a solution of the compound from Step G (394 mg, 1.0 mmol) in anhydrous pyridine (5 mL) was added p-methoxyphenylchlorodiphenylmethane (946 mg, 3.06 mmol) and 4-dimethylaminopyridine (DMAP) (123 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 20 h. It was then diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 15 mL) followed by water (2 x 15mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to an oil. The crude product was purified using column chromatography on silica gel eluting with 5% MeOH in CH_2Cl_2. The

appropriate fractions were collected and evaporated to give the title compound (540 mg).

^{1}H NMR (DMSO-d_6): δ 7.85 (s, 1H), 7.65 (s, 1H), 7.41 (d, 1H, J = 3.8Hz), 7.25-7.03 (m, 12H), 6.78 (d, 1H, J = 3.6 Hz), 6.69 (d, 2H, J = 9 Hz), 5.97 (s, 1H), 5.00-4.94 (m, 2H), 3.85-3.62 (m, 4H), 3.59 (s, 3H), 0.83 (s, 9H), 0.55 (s, 3H), 0.003 (s, 6H).

Step I: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[5-*O*-(*tert*-butyldimethylsilyl)-3-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-10)

To a solution of the compound from Step H (400 mg, 0.6 mmol) and anhydrous DMAP (73 mg, 0.6 mmol) in anhydrous CH_2Cl_2 (7 mL) was added slowly triethylamine (250 μL, 1.8 mmol). To the stirred solution was added octanoyl chloride (200 μL, 1.2 mmol) over 15 min. The reaction mixture was stirred for an additional 1.5 h. It was then diluted with methylene chloride (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 10 mL) and water (10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was subjected to column chromatography on silica gel eluting with 5% MeOH in CH_2Cl_2 to afford the title compound as a light yellow foam (340 mg).

^{1}H NMR (DMSO-d_6): δ 8.02 (s, 1H), 7.75 (s, 1H), 7.58 (d, 1H, J = 3.6 Hz), 7.34-7.05 (m, 12H), 7.02 (d, 1H, J = 3.6 Hz), 6.79 (d, 2H, J = 9.0 Hz), 6.01 (s, 1H), 5.61 (s, 1H), 5.34 (d, 1H, J = 9.0 Hz), 4.19-4.14 (m, 1H), 4.00-3.94 (m, 1H), 3.67-3.62 (m, 4H), 3.48-3.40 (m, 1H), 2.40-2.32 (m, 2H), 1.60-1.40 (m, 2H), 1.23 (bs, 8H), 0.91 (s, 9H), 0.84-0.80 (m, 3H), 0.67 (s, 3H), 0.07 (s, 6H).

Step J: 4-Amino-7-[5-*O*-(*tert*-butyldimethylsilyl)-3-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-11)

A solution of the compound from Step I (250 mg, 0.31 mmol) in 6:3:1 MeOH:acetic acid:H_2O (10 mL) was stirred at 50°C for 12 h. The reaction mixture was then concentrated to dryness. The residue was diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 15 mL) and water (2 x 10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The crude product (200 mg) was used without further purification in Step K below. Further purification of a small amount was accomplished by silica gel column chromatography using 5% MeOH in CH_2Cl_2 as the eluent to give the title compound as a white foam .

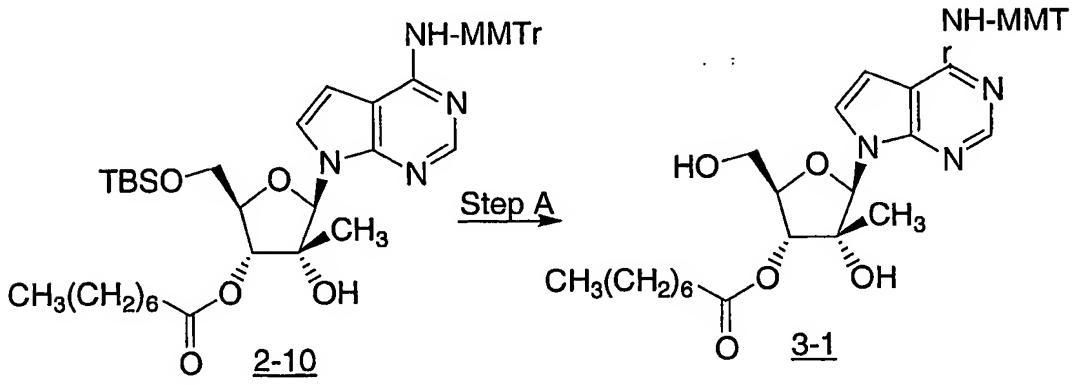
^{1}H NMR ($CDCl_3$): δ 8.29 (s,1H), 7.57 (d, 1H, J = 3.8 Hz), 6.37 (d, 1H, J = 3.8 HZ), 6.28 (s, 1H), 5.33-5.28 (m, 3H), 4.29-4.23 (m, 1H), 4.08-4.01 (m, 1H), 3.86-3.79 (m,1H), 2.45-2.37 (m, 2H), 1.69-1.62 (m, 2H), 1.29-1.23 (m,8H), 0.97-0.84 (m, 12H), 0.11 (s, 6H).

Step K: 4-Amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-12)

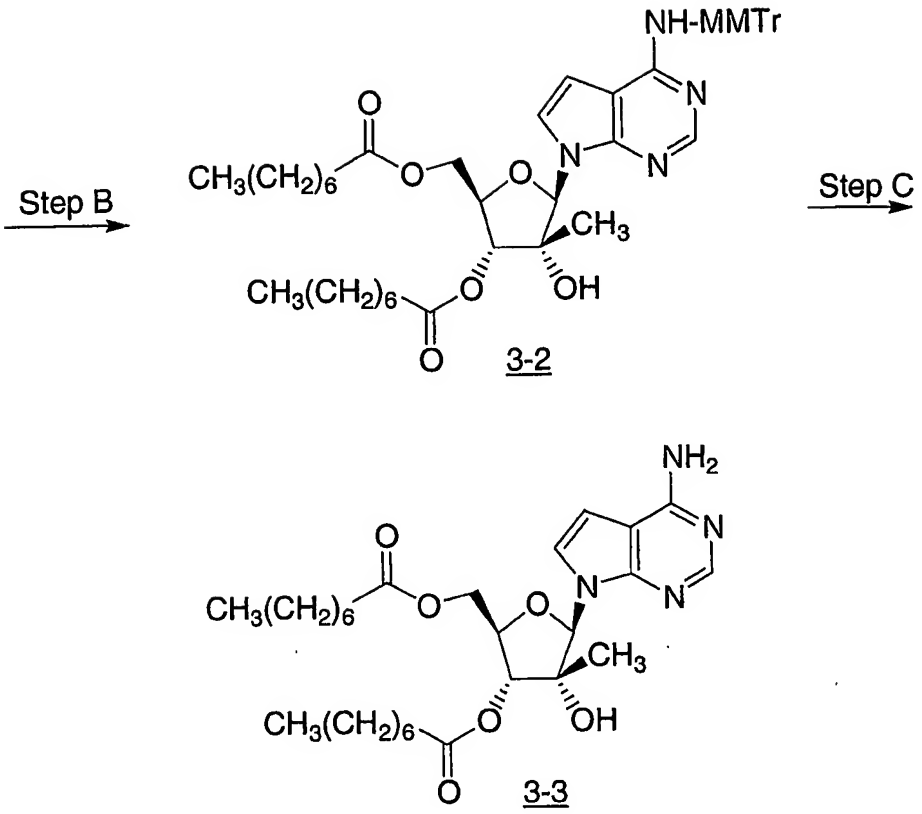
To a solution of the compound from Step J (230 mg, 0.44 mmol) in anhydrous THF (5mL), was added triethylamine (300 μL, 2.14 mmol) and triethylamine trihydrofluoride (750 μL, 4.5 mmol). The solution was stirred overnight at room temperature. The reaction mixture was then diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate (3 x 10 mL) and water (10 mL). After drying the organic layer over anhydrous sodium sulfate and filtration, the solvent was evaporated. The resulting oil was purified on a silica gel column eluting with 1:1 acetone/CH_2Cl_2 followed by 10% MeOH in CH_2Cl_2. The appropriate fractions were concentrated and lyophilized to afford the title compound as a colorless powder (90 mg).

^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 7.31 (d, 1H, J = 3.8 Hz), 6.39 (d, 1H, J = 3.8 Hz), 6.16 (s, 1H), 5.44 (d, 1H, J = 7.8 Hz), 5.23 (bs, 2H), 4.31-4.24 (m, 1H), 4.14-4.06 (m, 1H), 3.84-3.76 (m, 1H), 2.48-2.40 (m, 2H), 1.80-1.50 (m, 3H), 1.34-1.23 (m, 7H), 0.95 (s, 3H), 0.88-0.55 (m, 3H).

Scheme 3



(MMTr = p-methoxyphenyldiphenylmethyl)



EXAMPLE 39

4-Amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-3)

Step A: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[3-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-1)

A solution of the compound from Step I of Example 1 (1-10) (300 mg, 0.37 mmol), anhydrous triethylamine (300 μL, 2.14 mmol) and triethylamine trihydrofluoride (750 μL, 4.5 mmol) in anhydrous THF (5 mL) was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL) followed by water (2 x 15 mL). The organic layer was separated, dried over sodium sulfate, filtered, and evaporated. The crude product was purified on a silica gel column using 10-15% acetone in CH_2Cl_2 as the eluent. The appropriate fractions were combined and evaporated to afford the title compound as a colorless foam (240 mg).

^{1}H NMR (DMSO-d_6): δ 8.03 (s, 1H), 7.79 (s, 1H), 7.56 (d, 1H, J = 3.8 Hz), 7.38-7.17 (m, 12H), 7.04 (d, 1H, J = 3.8 Hz), 6.83 (d, 2H, J = 9.0 Hz), 6.13 (s, 1H), 5.56 (s, 1H), 5.31 (d, 1H, J = 9 Hz), 5.21-5.16 (m, 1H), 4.20-4.08 (m, 1H), 3.38-3.70 (m, 4H), 3.65-3.40 (m, 2H), 2.43-2.36 (m, 2H), 1.63-1.45 (m, 2H), 1.27 (bs, 8H), 0.91-0.84 (m, 3H), 0.74 (s, 3H).

Step B: 4-(p-Methoxyphenyldiphenylmethylamino)-7-[3,5-di-*O*-(1-oxo-octyl)-2-*C*-methyl-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-2)

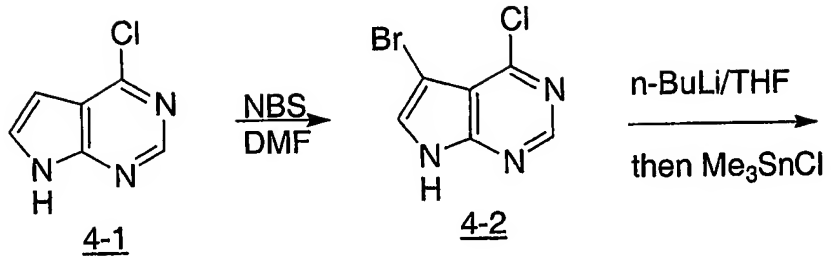
A solution of the compound from Step B (18 mg, 0.026 mmol) and DMAP (3.5 mg, 0.028 mmol) in anhydrous CH_2Cl_2 (300 μL) was cooled in an ice bath for 10 minutes under an argon atmosphere. To this solution was added triethylamine (7.5 μL, 0.053 mmol) followed by octanoyl chloride (6.6 μL, 0.038 mmol). The reaction mixture was stirred at this temperature for 2 h, diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous sodium bicarbonate solution (2 x 10 mL) followed by water (10 mL). The crude product obtained after evaporation was purified by column chromatography on silica gel eluting with 10% acetone in CH_2Cl_2. The title compound was obtained as a colorless foam (13.5 mg).

Step C: 4-Amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3-3)

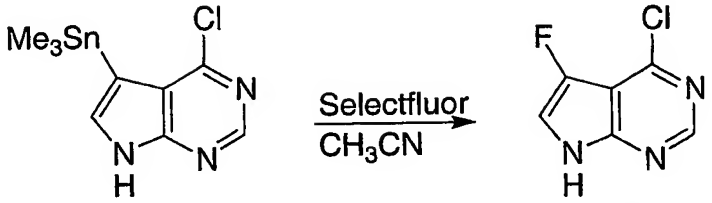
A solution of the compound from Step B (13 mg, 0.016 mmol) in 6:3:1 MeOH: acetic acid: H_2O (500 μL) was stirred at 50°C for 15 h. The reaction mixture was then concentrated to dryness. The residue was diluted with ethyl acetate (15 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 5 mL) and water (2 x 5 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The crude product was purified by silica gel column chromatography eluting with 10% acetone in dichloromethane to afford the title compound as a white foam (6.0 mg).

^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H), 7.25 (d, 1H, J = 3.4 Hz), 6.40 (d, 1H, J = 4.0 Hz), 6.23 (s, 1H), 5.22-5.39 (m, 3H), 4.60-4.39 (m, 4H), 2.47-2.35 (m, 4H), 1.82-1.60 (m, 4H), 1.27 (bs, 16 H), 0.87 (s, 3H), 0.873-0.80 (m, 6H).

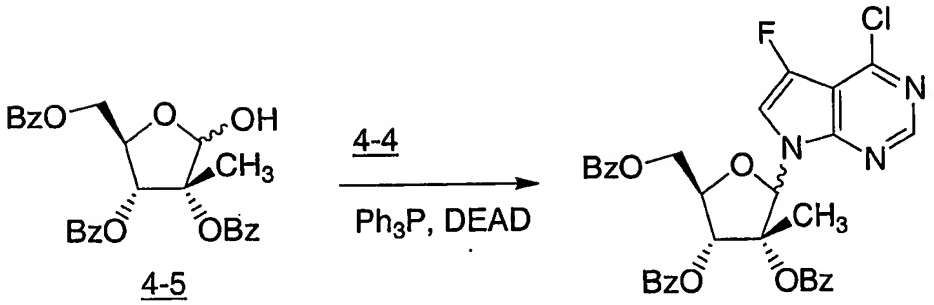
Scheme 4



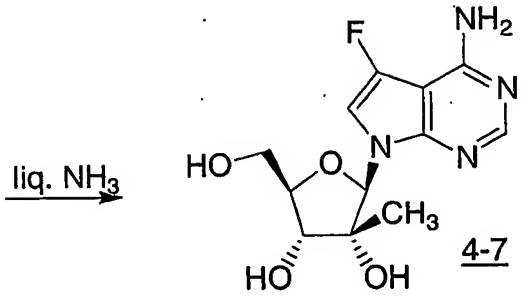
4-1 4-2



4-3 4-4



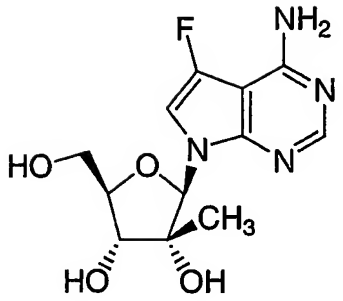
4-5 4-6



4-7

EXAMPLE 40

4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-7)



Step A: 5-Bromo-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-2)

To a solution of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-1) (1.53 g, 10.0 mmol) in DMF (20 mL) was added N-bromosuccinimide (1.78 g, 10.0 mmol) in DMF (10 mL) dropwise at 0°C. The reaction mixture was stirred at 0°C for 30 min and then at room temperature for 1 h. Methanol (25 mL) was added, and the reaction mixture was stirred for an additional 1 h. The solvent was evaporated and the residue was crystallized from methanol to give the title compound as white solid.

Step B: 5-(Trimethylstannyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-3)

To a solution of the compound from Step A (0.92 g, 4 mmol) in THF (25 mL) was added n-BuLi (2.5 M solution in hexane, 3.48 mL) dropwise at –78°C. After the addition, the reaction mixture was stirred at –78°C for an additional 30 min. To this solution was added trimethyltin chloride (0.88 g, 4.4 mmol) in THF (8 mL) dropwise for a period of 10 min. The reaction mixture was brought to room temperature slowly and stirred at room temperature overnight. Saturated aqueous ammonium chloride (60 mL) was added and extracted with ethyl acetate (3 x 70 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel to give the title compound as a colorless solid.

Step C: 5-Fluoro-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-4)

To a solution of the compound from Step B (1.97 g, 6.20 mmol) in CH_3CN (60 mL) was added [1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)] (SELECTFLUOR® fluorinating

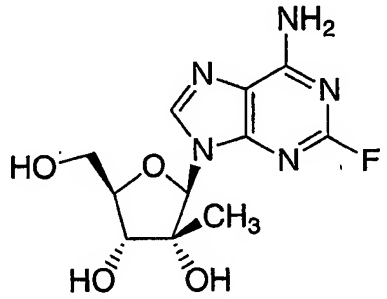
reagent) (2.40 g, 6.5 mmol) in one portion and the reaction mixture was stirred at room temperature for 7 h. The white precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was purified over silica gel using ethyl acetate/hexane (3:7) as the eluent. Fractions containing the product were pooled and eveporated in vacuo to give the title compound as a colorless solid.
^{1}H-NMR (MeOH-d_4): δ 8.53 (s, 1H), 7.37 (d, J = 2.8 Hz);
^{19}F-NMR (DMSO-d_6): δ -171.5.

Step D: 4-Amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (4-7)

To a solution of the compound from Step C (0.075 g, 0.44 mmol), 2,3,5-tri-O-benzoyl-2-*C*-methyl-D-ribofuranose [Harry-O'kuru, Rogers E.; Smith, Jennifer M.; Wolfe, Michael S, "A Short, Flexible Route toward 2'-*C*-Branched Ribonucleosides," J. Org. Chem.,62: 1754-1759 (1997)] (4-5) (0.25 g, 0.53 mmol) and triphenylphosphine (0.23 g, 0.88 mmol) in THF (15 mL) was added diethyl azodicarboxylate (DEAD) (0.14 mL, 0.88 mmol). The reaction mixture was stirred at room temperature overnight. The solution was directly adsorbed onto silica gel and purified over silica gel using ethyl acetate/hexane 1:9 as the eluent Appropriate fractions were dissolved in dioxane (3 mL) and liquid ammonia (4 mL) and the mixture was heated in a steel bomb at 85°C for 24 h. The solvent was evaporated and the residue was purified over silica gel using methanol/dichloromethane (1:9) as the eluent. Fractions containing the desired compound were pooled and evaporated in vacuo to give the title compound.
^{1}H-NMR (MeOH-d_4): δ 8.07 (s, 1H), 7.41 (d, J = 2.2 Hz, 1H), 6.25 (d, J = 1.8 Hz), 4.09-3.95 (m, 3H), 3.82 (dd, J = 2.7, 12.5 Hz, 1H);
^{19}F-NMR (MeOH-d_4): δ -170.4; mass spectrum: 321 $(M+Na)^+$.

EXAMPLE 41

6-Amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine



Step A: 2-Amino-6-chloro-9-(2,3,5-tri-O-benzoyl-2-*C*-methyl-β-D-ribofuranosyl)purine

To a pre-cooled solution of 1,2,3,5-tetra-O-benzoyl-2-*C*-methyl-D-ribofuranose [Harry-O'kuru, Rogers E.; Smith, Jennifer M.; Wolfe, Michael S, "A Short, Flexible Route toward 2'-*C*-Branched Ribonucleosides," J. Org. Chem.,62: 1754-1759 (1997)] (1.74 g, 3.00 mmol) in acetonitrile (15 mL) was added 2-amino-6-chloropurine (0.56 g, 3.30 mmol), then diazabicyclo[5.4.0]undec-7-ene (DBU) (1.37 g, 9.00 mmol), and then dropwise trimethylsilylmethyl trifluoromethanesulfonate (TMS trifate) (2.67 g, 12.00 mmol). The resulting mixture was heated to 65°C for 4 h, then cooled and partitioned between saturated aqueous sodium bicarbonate (200 mL) and dichloromethane (200 mL). The organic phase was dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude product was used directly in step B.

Step B: 2-Amino-6-chloro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine

To the crude compound from Step A (2.54 g) in THF (18 mL) was added aqueous 2N LiOH (6 mL). The resulting mixture was stirred at room temperature for 3 h, the THF evaporated in vacuo and the resulting aqueous phase neutralized by addition of aqueous 2N hydrochloric acid. The mixture was adsorbed onto silica gel by evaporation in vacuo and purified on silica gel using

methanol/dichloromethane (1:4) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product as a colorless powder.

Step C: 2,6-Diamino-9-(2-*C*-methyl-β-D-ribofuranosyl)purine

To the compound from Step B (100 mg) was added ammonium hydroxide (5 mL) and the resulting mixture was stirred at 80°C in a Parr bomb overnight. The mixture was cooled and evaporated in vacuo and adsorbed onto silica and purified on silica gel using methanol/dichloromethane (1:4) as the eluent. Fractions containing the product were combined and evaporated in vacuo to give the desired product as a colorless powder.

Step D: 6-Amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine

To a mixture of HF/pyridine (70%) (1 mL) in pyridine (1 mL) at -30°C was added the compound from Step C (29.6 mg, 0.10 mmol) in pyridine (0.5 mL), followed by addition of tert-butyl nitrite (0.018 mL, 0.15 mmol). The mixture was stirred for 5 minutes and then poured into ice water (5 mL), neutralized with 2N NaOH and evaporated in vacuo. The crude product was purified on silica gel using methanol/dichloromethane (1:9 through 1:4) as the eluent. Fractions containing the desired product were pooled and evaporated in vacuo to give the desired compound as a colorless oil.

^{1}H-NMR (acetonitrile-d_3): δ 8.23 (s, 1H), 5.93 (s, 1H), (t, J = 8.4 Hz, 1H), 4.00 (m, 2H), 3.81 (m, 1H), 3.70 (s, 1H), 3.60 (m, 1H), 0.90 (s, 3H).

^{19}F-NMR (MeOH-d_4): -80;

Mass spectrum: 298.3 $(M-H)^+$ and 597.1 $(2M-H)^+$.

BIOLOGICAL ASSAYS

The assays employed to measure activity against vaccinia virus are described below:

a. Determination of In Vitro Antiviral Activity of Compounds Against Vaccinia Virus (Cytopathic Effect Inhibition Assay):

Assay conditions are described in the article by Sidwell and Huffman, "Use of disposable microtissue culture plates for antiviral and interferon induction studies," Appl. Microbiol. 22: 797-801 (1971).

Vaccinia virus, Lederle strain from the ATCC, was used with Vero cells and media (9% fetal bovine serum, 0.1% $NaHCO_3$, no antibiotics) as stated in the article. Antiviral test medium was MEM with 2% FBS and 0.18% $NaHCO_3$.

Four and seven point titrations were used to assay compound inhibition. Final compound concentrations for 4-point titrations were 100, 10, 1, and 0.1 μM. Seven-point titrations were performed by preparing one-half log serial dilutions from maximum compound concentrations of either 100 μM or 320 μM. Virus was added to the assay plate approximately 5 min after the test compound. Assay plates were incubated with humidified air and 5% CO_2 at 37°C. Cytotoxicity was monitored in the control cells microscopically for morphologic changes. Regression analysis of the virus CPE data and the toxicity control data gave the ED50 (50% effective dose) and CC50 (50% cytotoxic concentration). The selectivity index (SI) was calculated by the formula: SI = CC50 ÷ ED50.

(S)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine (cidofovir) was used as a positive control for anti-vaccinia virus testing.

Representative compounds tested in the anti-vaccinia virus assay exhibited EC_{50}'s less than 100 micromolar.

b. Determination of In Vitro Antiviral Activity of Compounds Against Vaccinia Virus (Neutral Red Uptake Assay)

After performing the CPE inhibition assay above, an additional cytopathic detection method was used. McManus described the detection method in "Microtiter Assay for Interferon: Microspectrophotometric Quantitation of Cytopathic Effect," Appl. Environ. Microbiol., 31: 35-38 (1976). A Model EL309 microplate reader (Bio-Tek Instruments Inc.) was used at 540 nm to directly read the assay plate. ED50 and CD50 were calculated as above.

The nucleoside derivatives of the present invention were also evaluated for cellular toxicity and antiviral specificity in the counterscreens described below.

COUNTERSCREENS:

The ability of the nucleoside derivatives of the present invention to inhibit human DNA polymerases was measured in the following assays.

a. Inhibition of Human DNA Polymerases alpha and beta:

Reaction Conditions:
50 μL reaction volume

Reaction buffer components:
20 mM Tris-HCl, pH 7.5
200 μg/mL bovine serum albumin
100 mM KCl
2 mM β-mercaptoethanol
10 mM $MgCl_2$
1.6 μM dATP, dGTP, dCTP, dTTP
α-^{33}P-dATP

Enzyme and template:
0.05 mg/mL gapped fish sperm DNA template
0.01 U/μL DNA polymerase α or β

Preparation of gapped fish sperm DNA template:
Add 5 μL 1M $MgCl_2$ to 500 μL activated fish sperm DNA (USB 70076);
Warm to 37°C and add 30 μL of 65 U/μL of exonuclease III (GibcoBRL 18013-011);
Incubate 5 min at 37°C;
Terminate reaction by heating to 65 °C for 10 min;
Load 50-100 μL aliquots onto Bio-spin 6 chromatography columns (Bio-Rad 732-6002) equilibrated with 20 mM Tris-HCl, pH 7.5;
Elute by centrifugation at 1,000Xg for 4 min;
Pool eluate and measure absorbance at 260 nm to determine concentration.

The DNA template was diluted into an appropriate volume of 20 mM Tris-HCl, pH 7.5 and the enzyme was diluted into an appropriate volume of 20 mM Tris-HCl, containing 2 mM β-mercaptoethanol, and 100 mM KCl. Template and enzyme were pipetted into microcentrifuge tubes or a 96 well plate. Blank reactions excluding enzyme and control reactions excluding test compound were also prepared using enzyme dilution buffer and test compound solvent, respectively. The reaction was initiated with reaction buffer with components as listed above. The reaction was incubated for 1 h at 37°C. The reaction was quenched by the addition of 20 μL 0.5M

EDTA. 50 μL of the quenched reaction was spotted onto Whatman DE81 filter disks and air dried. The filter disks were repeatedly washed with 150 mL 0.3M ammonium formate, pH 8 until 1 mL of wash is < 100 cpm. The disks were washed twice with 150 mL absolute ethanol and once with 150 mL anhydrous ether, dried and counted in 5 mL scintillation fluid.

The percentage of inhibition was calculated according to the following equation: % inhibition = [1-(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

b. Inhibition of Human DNA Polymerase gamma :

The potential for inhibition of human DNA polymerase gamma was measured in reactions that included 0.5 ng/ μL enzyme; 10 μM dATP, dGTP, dCTP, and TTP; 2 μCi/reaction [α-^{33}P]-dATP, and 0.4 μg/μL activated fish sperm DNA (purchased from US Biochemical) in a buffer containing 20 mM Tris pH8, 2 mM β-mercaptoethanol, 50 mM KCl, 10 mM $MgCl_2$, and 0.1 μg/μL BSA. Reactions were allowed to proceed for 1 h at 37°C and were quenched by addition of 0.5 M EDTA to a final concentration of 142 mM. Product formation was quantified by anion exchange filter binding and scintillation counting. Compounds were tested at up to 50 μM.

The percentage of inhibition was calculated according to the following equation: % inhibition = [1-(cpm in test reaction - cpm in blank)/(cpm in control reaction - cpm in blank)] x 100.

The ability of the purine nucleoside derivatives of the present invention to inhibit HIV infectivity and HIV spread was measured in the following assays.

c. HIV Infectivity Assay

Assays were performed with a variant of HeLa Magi cells expressing both CXCR4 and CCR5 selected for low background β-galactosidase (β-gal) expression. Cells were infected for 48 h, and β-gal production from the integrated HIV-1 LTR promoter was quantified with a chemiluminescent substrate (Galactolight Plus, Tropix, Bedford, MA). Inhibitors were titrated (in duplicate) in twofold serial dilutions starting at 100 μM; percent inhibition at each concentration was calculated in relation to the control infection.

d. Inhibition of HIV Spread

The ability of the compounds of the present invention to inhibit the spread of the human immunedeficiency virus (HIV) was measured by the method described in U.S. Patent No. 5,413,999 (May 9, 1995), and J.P.Vacca, et al., Proc. Natl. Acad. Sci., 91: 4096-4100 (1994), which are incorporated by reference herein in their entirety.

The nucleoside derivatives of the present invention were also screened for cytotoxicity against cultured hepatoma (HuH-7) cells containing a subgenomic HCV Replicon in an MTS cell-based assay as described in the assay below. The HuH-7 cell line is described in H. Nakabayashi, et al., Cancer Res., 42: 3858 (1982).

e. Cytotoxicity assay:

Cells were plated at 15-20,000 cells/well in 100 μL of appropriate media and incubated 18 h at 37°C, 5% CO_2. 100 μL of compound diluted in complete media was added to the cells for a final of 1% DMSO concentration. The plates were incubated at 37°C and 5% CO_2 for 24 h. After the incubation period, 40 μL of CellTiter 96 Aqueous One Solution Cell Proliferation Assay reagent (MTS) (Promega) was added to each well, and the plates were incubated at 37°C and 5% CO_2 for 1 h. The plates were agitated to mix well and absorbance at 490 nm was read using a plate reader. Metabolically active cells reduce MTS to formazan. Formazan absorbs at 490 nm. The absorbance at 490 nm in the presence of compound was compared to absorbance in cells without any compound added.

Reference: Cory, A. H. *et al.*, "Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture," Cancer Commun. 3: 207 (1991).

EXAMPLE OF A PHARMACEUTICAL FORMULATION

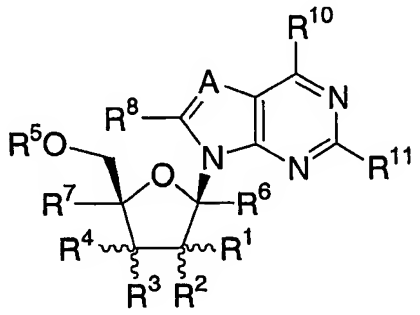
As a specific embodiment of an oral composition of a compound of the present invention, 50 mg of the compound of Example 2 is formulated with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size O hard gelatin capsule.

While the invention has been described and illustrated in reference to specific embodiments thereof, those skilled in the art will appreciate that various changes, modifications, and substitutions can be made therein without departing from

the spirit and scope of the invention. For example, effective dosages other than the preferred doses as set forth hereinabove may be applicable as a consequence of variations in the responsiveness of the human being treated for severity of the HCV infection. Likewise, the pharmacologic response observed may vary according to and depending upon the particular active compound selected or whether there are present pharmaceutical carriers, as well as the type of formulation and mode of administration employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended therefore that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

WHAT IS CLAIMED IS:

1. A method of inhibiting orthopoxvirus replication comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of structural formula I:



(I)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{2-4} alkenyl, C_{2-4} alkynyl, or C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^2 is amino, fluorine, hydroxy, C_{1-10} alkylcarbonyloxy, mercapto, or C_{1-4} alkoxy;

R^3 and R^4 are each independently selected from the group consisting of hydrogen, cyano, azido, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, C_{2-18} alkenylcarbonyloxy, C_{1-10} alkyloxycarbonyloxy, C_{3-6} cycloalkylcarbonyloxy, C_{3-6} cycloalkyloxycarbonyloxy, mercapto, amino, C_{1-4} alkoxy, C_{2-4} alkenyl, C_{2-4} alkynyl, and C_{1-4} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-4} alkoxy, C_{1-4} alkylthio, or one to three fluorine atoms;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, C_{2-18} alkenylcarbonyl, C_{1-10} alkyloxycarbonyl, C_{3-6} cycloalkylcarbonyl, C_{3-6} cycloalkyloxycarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or $P(O)R^{13}R^{14}$;

R^6 and R^7 are each independently hydrogen, methyl, hydroxymethyl, or fluoromethyl;

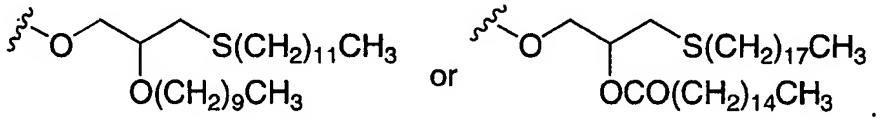
R^8 is hydrogen, C_{1-4} alkyl, C_{2-4} alkynyl, halogen, cyano, carboxy, C_{1-4} alkyloxycarbonyl, azido, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, hydroxy, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfonyl, or (C_{1-4} alkyl)$_{0-2}$ aminomethyl;

R^9 is hydrogen, cyano, nitro, $NHCONH_2$, $CONR^{12}R^{12}$, $CSNR^{12}R^{12}$, $COOR^{12}$, $C(=NH)NH_2$, hydroxy, C_{1-3} alkoxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, halogen, or C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with one to three groups independently selected from halogen, amino, hydroxy, carboxy, and C_{1-3} alkoxy;

R^{10} and R^{11} are each independently hydrogen, hydroxy, mercapto, halogen, C_{1-4} alkoxy, C_{1-4} alkylthio, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{3-6} cycloalkylamino, di(C_{3-6} cycloalkyl)amino, phenyl-C_{1-2} alkylamino, C_{1-4} acylamino, C_{1-8} alkylcarbonyloxy, or $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl;

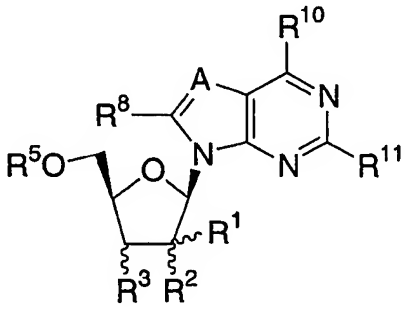
each R^{12} is independently hydrogen or C_{1-6} alkyl; and

R^{13} and R^{14} are each independently hydroxy, $OCH_2CH_2SC(=O)C_{1-4}$ alkyl, $OCH_2O(C=O)OC_{1-4}$ alkyl, $NHCHMeCO_2Me$, $OCH(C_{1-4}$ alkyl$)O(C=O)C_{1-4}$ alkyl,



2. A method of treating orthopoxvirus infection in a mammal in need thereof comprising administering a therapeutically effective amount of a compound of Claim 1.

3. The method of Claim 1 wherein the compound is of structural formula II:



(II)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;
R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or C_{1-3} alkoxy;
R^3 is hydrogen, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or C_{1-3} alkoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;
R^8 is hydrogen, amino, or C_{1-4} alkylamino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

4. The method of Claim 3 wherein
R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;
R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or methoxy;
R^3 is hydrogen, fluoro, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or methoxy;
R^5 is hydrogen, C_{1-16} alkylcarbonyl, or $P_3O_9H_4$;
R^8 is hydrogen or amino;
R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and
R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

5. The method of Claim 4 wherein the compound is selected from the group consisting of:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile,

4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-hydroxypurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-cyclopropylaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2,6-diaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-methylaminopurine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

6. The method of Claim 5 wherein the compound is selected from the group consisting of:
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,

4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

7. The method of Claim 6 wherein the compound is 4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

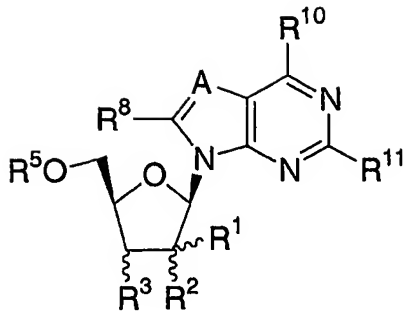
8. The method of Claim 6 wherein the compound is 4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

9. The method of Claim 6 wherein the compound is 4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

10. The method of Claim 6 wherein the compound is 4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

11. The method of Claim 6 wherein the compound is 4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

12. The method of Claim 2 wherein the compound is of structural formula II:



(II)

or a pharmaceutically acceptable salt thereof; wherein

A is N or C-R^9;

R^1 is C_{1-3} alkyl, wherein alkyl is unsubstituted or substituted with hydroxy, amino, C_{1-3} alkoxy, C_{1-3} alkylthio, or one to three fluorine atoms;

R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or C_{1-3} alkoxy;

R^3 is hydrogen, halogen, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or C_{1-3} alkoxy;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, $P_3O_9H_4$, $P_2O_6H_3$, or PO_3H_2;

R^8 is hydrogen, amino, or C_{1-4} alkylamino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

13. The method of Claim 12 wherein

R^1 is methyl, fluoromethyl, hydroxymethyl, difluoromethyl, trifluoromethyl, or aminomethyl;

R^2 is hydroxy, C_{1-16} alkylcarbonyloxy, fluoro, or methoxy;

R^3 is hydrogen, fluoro, hydroxy, C_{1-16} alkylcarbonyloxy, amino, or methoxy;

R^5 is hydrogen, C_{1-16} alkylcarbonyl, or $P_3O_9H_4$;

R^8 is hydrogen or amino;

R^9 is hydrogen, cyano, methyl, halogen, or $CONH_2$; and

R^{10} and R^{11} are each independently hydrogen, halogen, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, or C_{3-6} cycloalkylamino.

14. The method of Claim 13 wherein the compound is selected from the group consisting of:

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,

4-methylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-dimethylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-hydroxymethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide,
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2,4-diamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-4-cyclopropylamino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
2-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-hydroxypurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-cyclopropylaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2,6-diaminopurine,
9-(2-*C*-methyl-β-D-ribofuranosyl)-2-amino-6-methylaminopurine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

15. The method of Claim 14 wherein the compound is selected from the group consisting of:

4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-methyl-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-bromo-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-chloro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
4-amino-2-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine,
6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine,
2'-*C*-methyl-adenosine,
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine, and
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
and the corresponding 5'-triphosphates;
or a pharmaceutically acceptable salt thereof.

16. The method of Claim 15 wherein the compound is
4-amino-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

17. The method of Claim 15 wherein the compound is
4-amino-7-[2-*C*-methyl-3-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

18. The method of Claim 15 wherein the compound is
4-amino-7-(2-*C*-fluoromethyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

19. The method of Claim 15 wherein the compound is
4-amino-7-[2-*C*-methyl-3,5-di-*O*-(1-oxo-octyl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine;
or a pharmaceutically acceptable salt thereof.

20. The method of Claim 15 wherein the compound is 4-amino-5-fluoro-7-(2-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; or a pharmaceutically acceptable salt thereof.

21. The method of Claim 1 wherein said orthopoxvirus replication is vaccinia virus or variola virus replication.

22. The method of Claim 2 wherein said orthopoxvirus infection is vaccinia virus or variola virus infection.

23. The method of Claim 22 in combination with a therapeutically effective amount of another agent active against orthopoxvirus.

24. The method of Claim 23 wherein said agent active against orthopoxvirus is cidofovir, ribavirin, levovirin, or viramidine.

25. Use of a compound of Claim 1 for the inhibition of orthopoxvirus replication in a mammal.

26. Use of a compound of Claim 1 for the treatment of orthopoxvirus infection in a mammal.

27. The use of Claim 26 wherein said orthopoxvirus infection is vaccinia virus infection or variola virus infection.

28. Use of a compound of Claim 1 in the manufacture of a medicament for the inhibition of orthopoxvirus replication in a mammal.

29. Use of a compound of Claim 1 in the manufacture of a medicament for the treatment of orthopoxvirus infection in a mammal.

30. The use of Claim 29 wherein said orthopoxvirus infection is vaccinia virus or variola virus infection.

31. The method of Claim 8 wherein the orthopoxvirus replication is vaccinia virus or variola virus replication.

32. The method of Claim 17 wherein the orthopoxvirus replication is vaccinia virus or variola virus replication.

33. A compound which is 6-amino-2-fluoro-9-(2-*C*-methyl-β-D-ribofuranosyl)purine or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/03703

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/7052, 31/7076, 31/708
US CL : 514/43, 45, 46, 47, 48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/43, 45, 46, 47, 48; 536/27.1, 27.13, 27.14, 27.2, 27.21, 27.22, 27.23, 27.3, 27.4, 27.6, 27.61, 27.63, 27.7, 27.8, 27.81

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN (CAS ONLINE, MEDLINE, BIOSIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 01/90121 A2 (NOVIRIO PHARMACEUTICALS LIMITED) 29 November 2001 (29.11.2001), entire document.	1-33
Y	WO 01/92282 A2 (NOVIRIO PHARMACEUTICALS LIMITED) 06 December 2001 (06.12.2001), entire document.	1-33
Y,P	WO 02/18404 A2 (F. HOFFMANN-LA ROCHE AG) 07 March 2002 (07.03.2002), entire document.	1-33
Y,P	WO 02/32920 A2 (PHARMASSET LIMITED) 25 April 2002 (25.04.2002), entire document.	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2003 (11.07.2003)	24 JUL 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer James O. Wilson Telephone No. (703) 308-1235

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